Exanthematous drug eruptions

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Disclosure slide

- **Owner** of **adr-ac GmbH**, a company devoted to drug hypersensitivity analysis and research
- **Consultant** for Pfizer, Hoffmann LaRoche, Böhringer-Ingelheim, Novartis, Menarini, Aicuris, Swatch
- **Research support** by Swiss National Science foundation, Swiss Center of Applied Toxicology, Ulrich Müller Gierok Foundation, Pfizer, Hoffmann-LaRoche, Menarini

Exanthematous drug eruptions

- ~~«rashes»~~
- ~~Urticaria immediate~~ reactions
- Delayed appearing exanthems

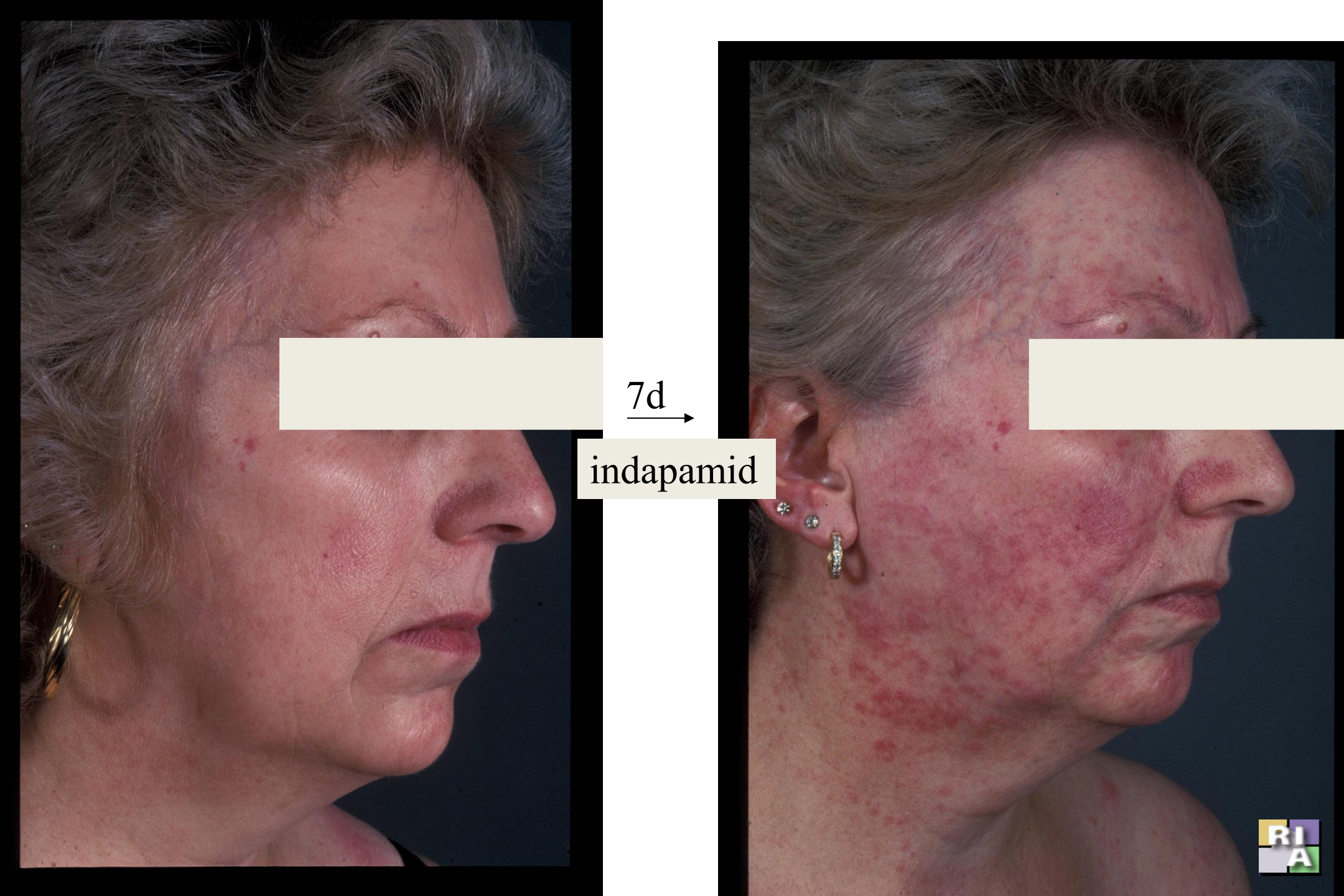
  with cell infiltration

  it is frequent - antibiotics (0.5 - 8% of treated)

  - antiepileptics

  - allopurinol, diuretics,

    antivirals, ...


7d
indapamid

Drug allergy

1) **What has happened ?**

history
drug exposure
cofactors
viral infection
previous drug allergies

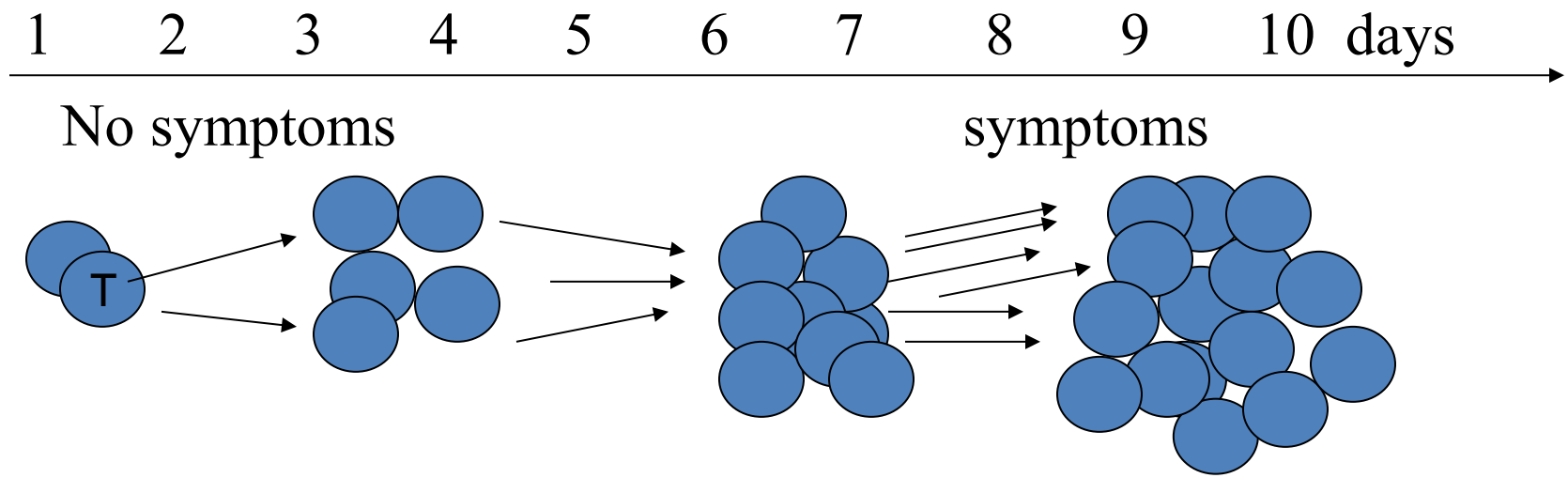
2) **Mechanism & how severe is the drug induced illness ?**

Danger signs:
timing
clinical
laboratory

3) **Which drug is responsible ?**

Skin testing
lymphocyte stimulation tests
provocation tests (?)

Delayed reaction: it is T-cell mediated



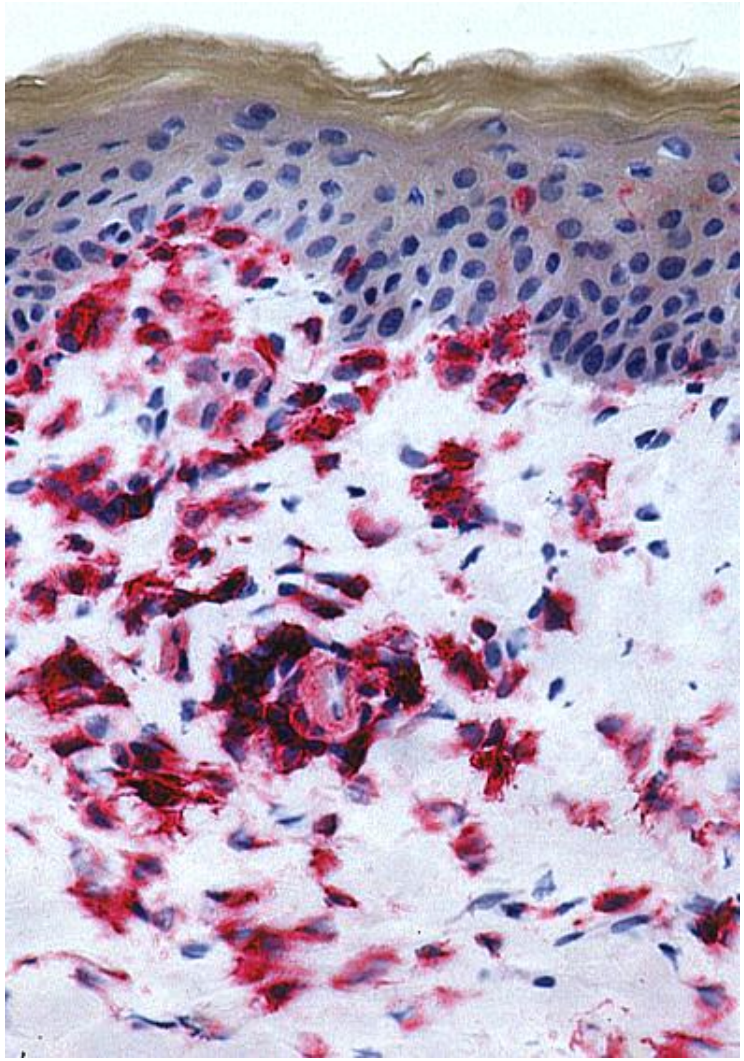
Few precursor cells

… Expansion….

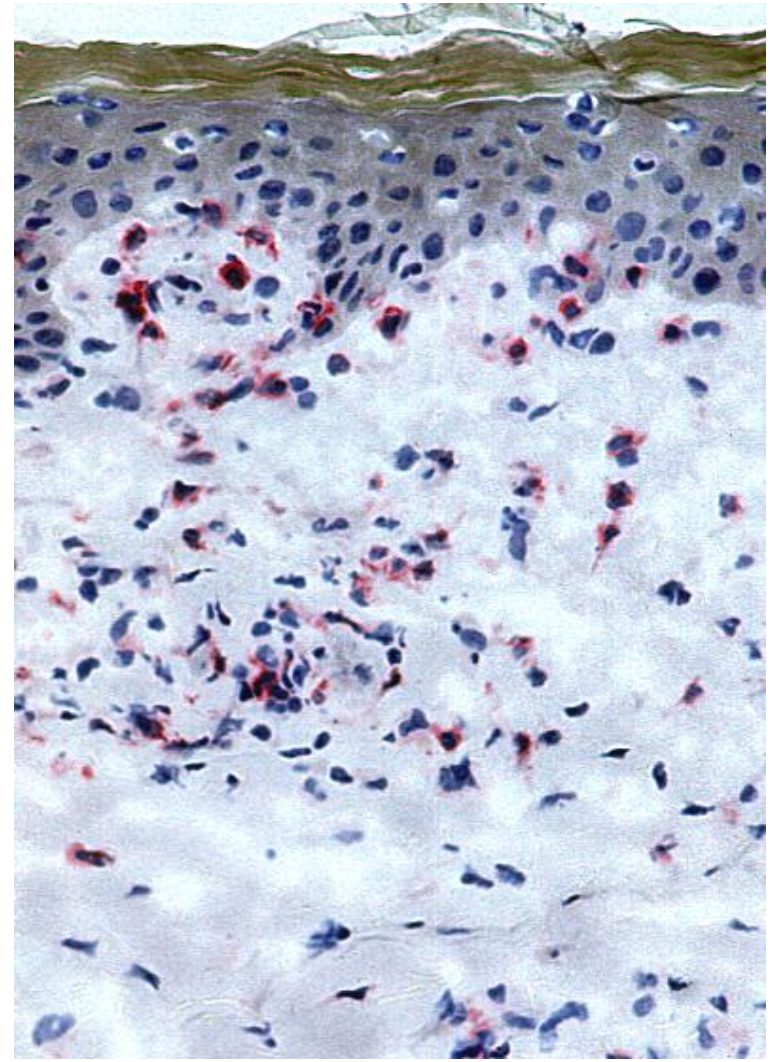
Symptoms arise if a certain amount of specific T-cells is homing to the tissue and exerts effector function

Maculo**papular** drug exanthem

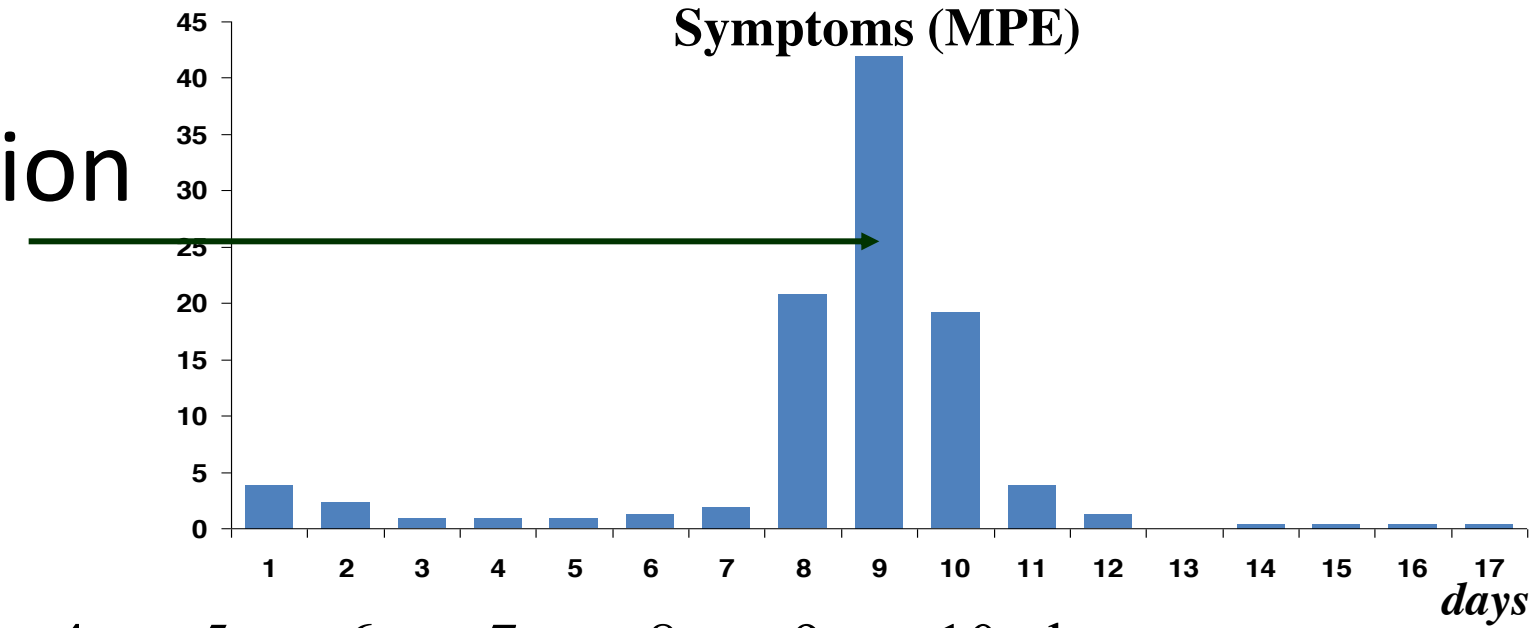
CD4+

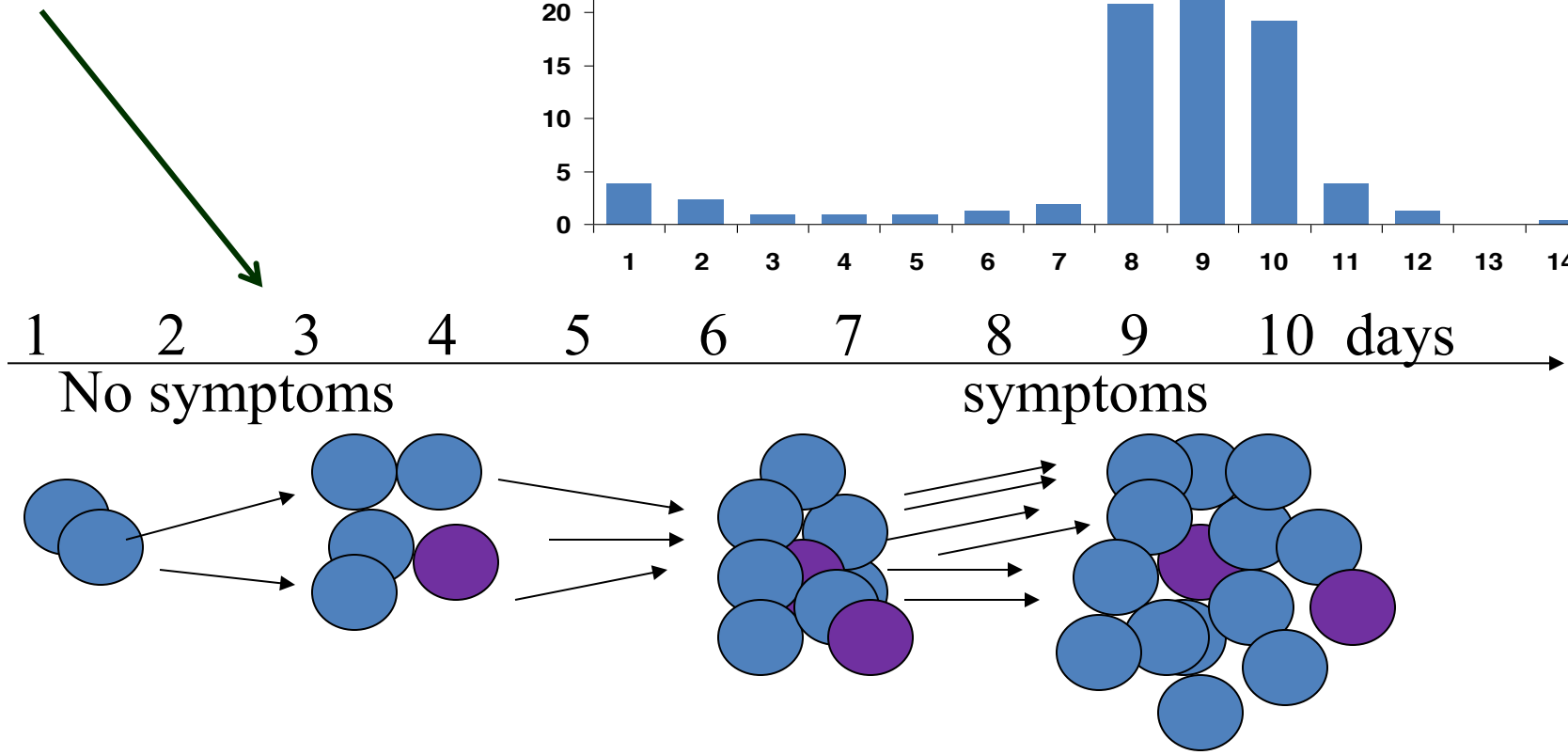


CD8 +



Delayed reaction



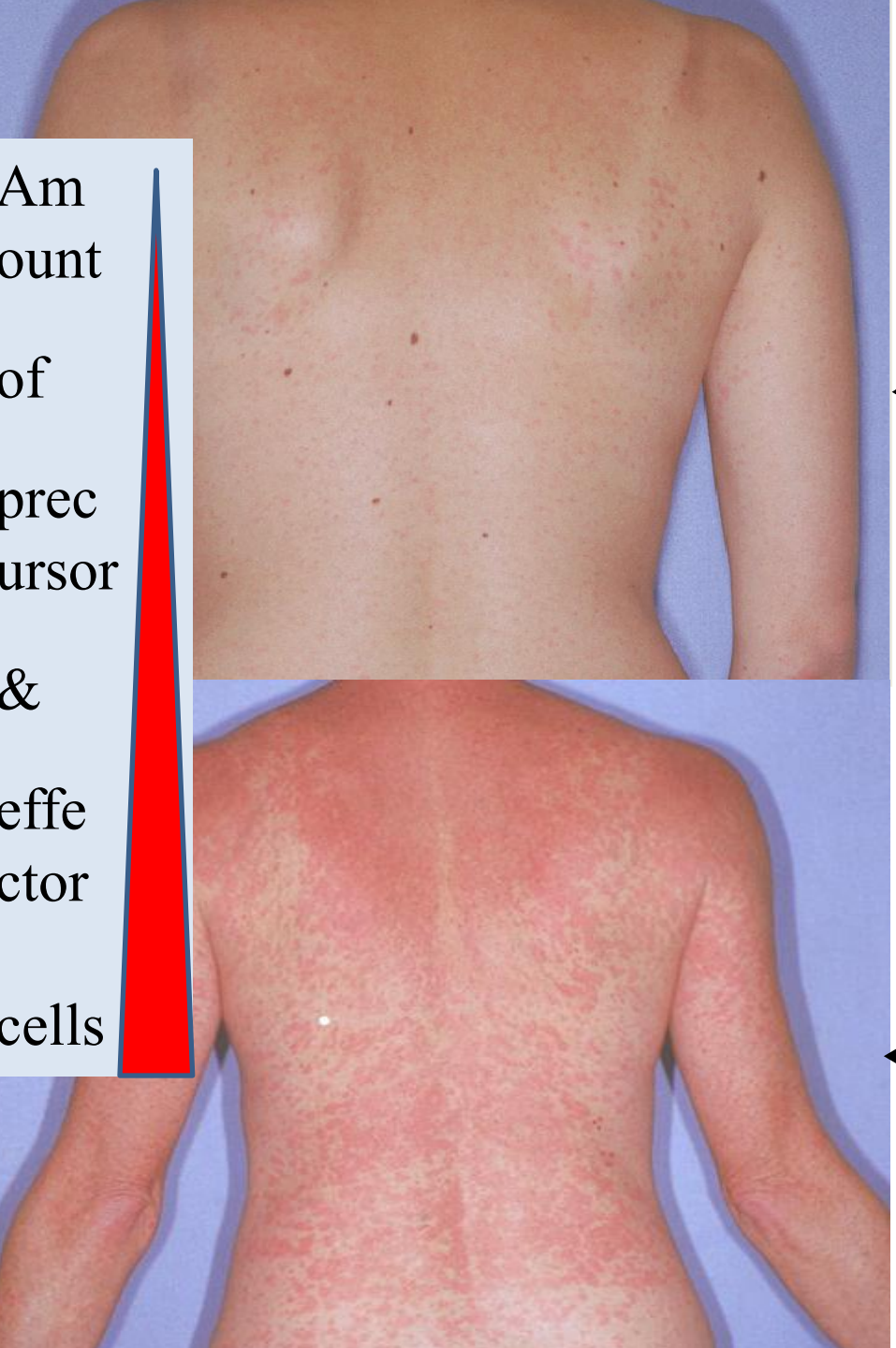


Few precursor cells

… Expansion….

Symptoms arise if a certain **amount** of specific T-cells is homing to the tissue and exerts **effector function**

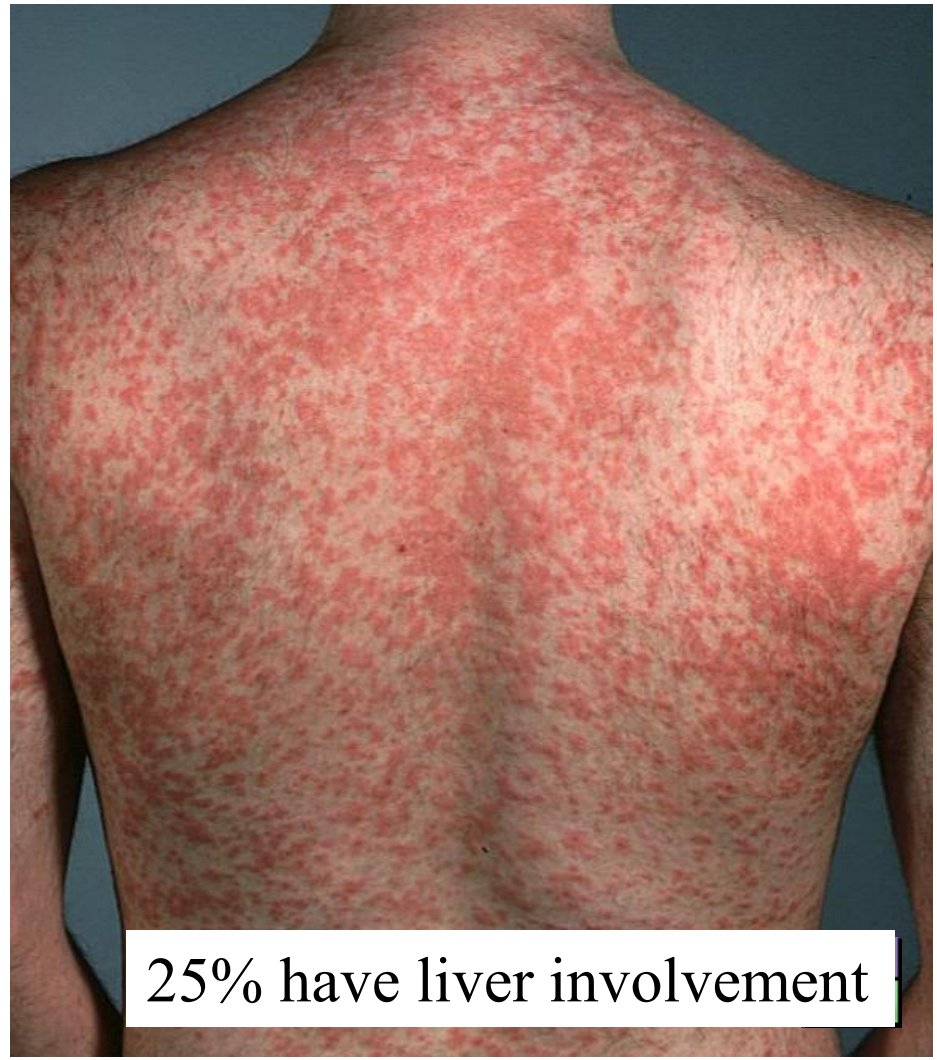
maculopapular exanthema



mild

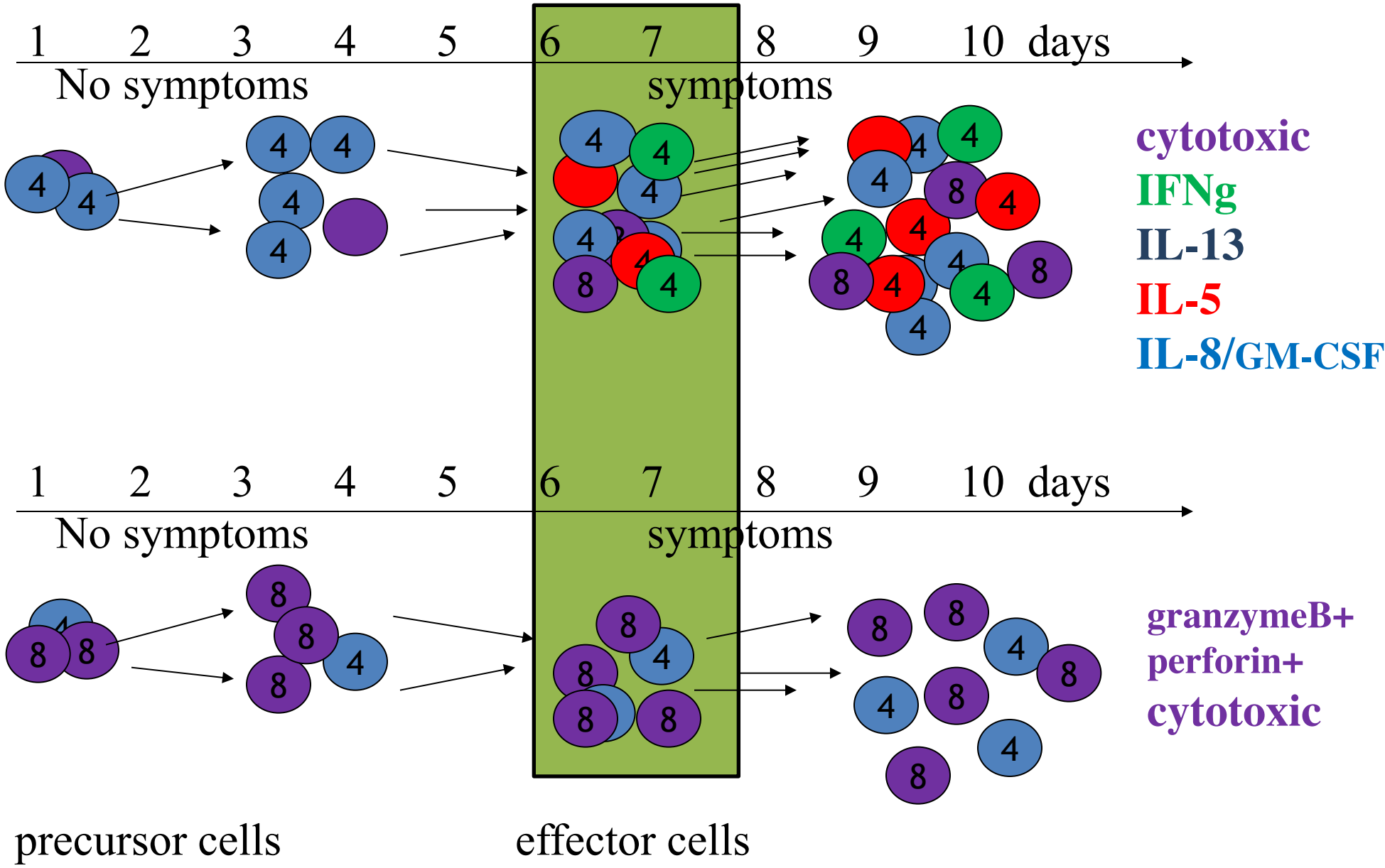
moderate

massive, confluent ↓



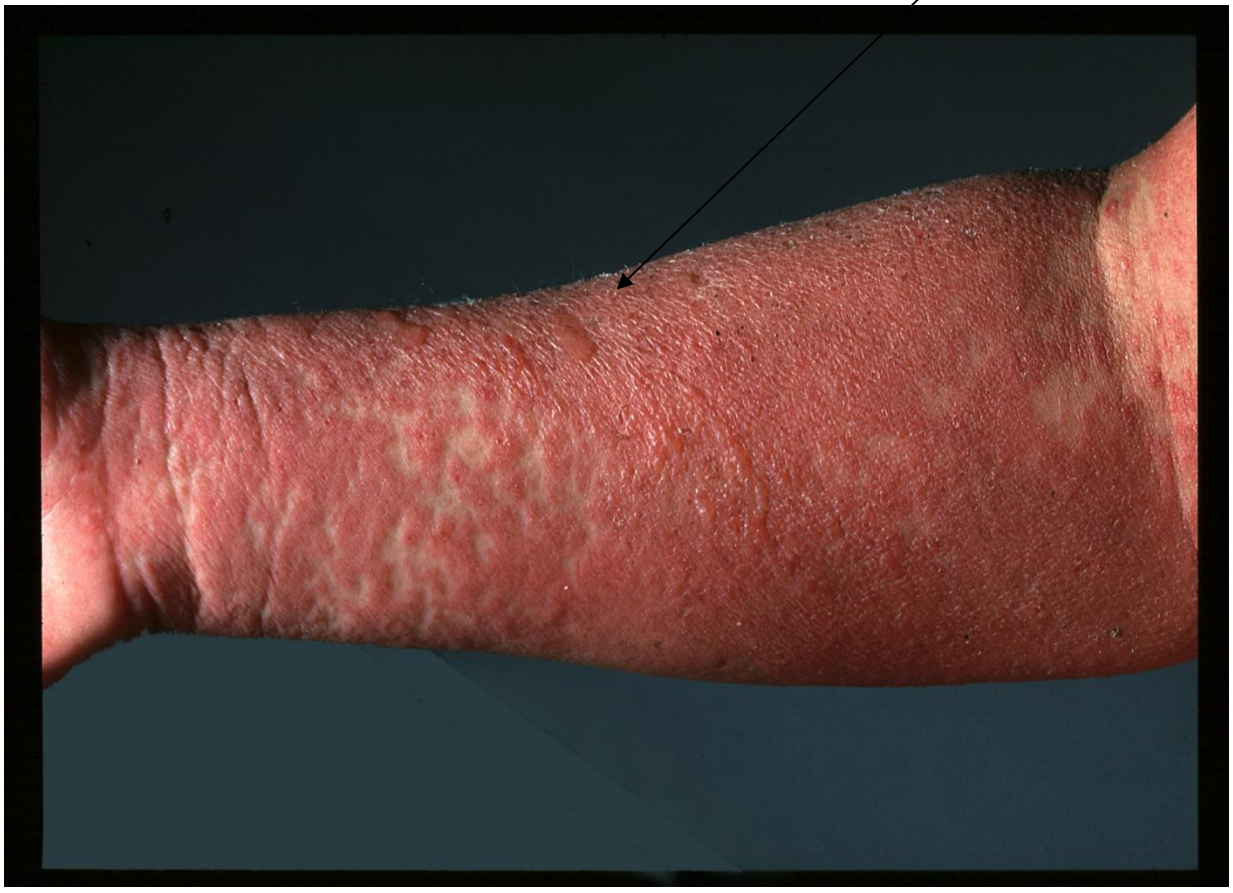
The function of effector T cells determines clincal phenotype:

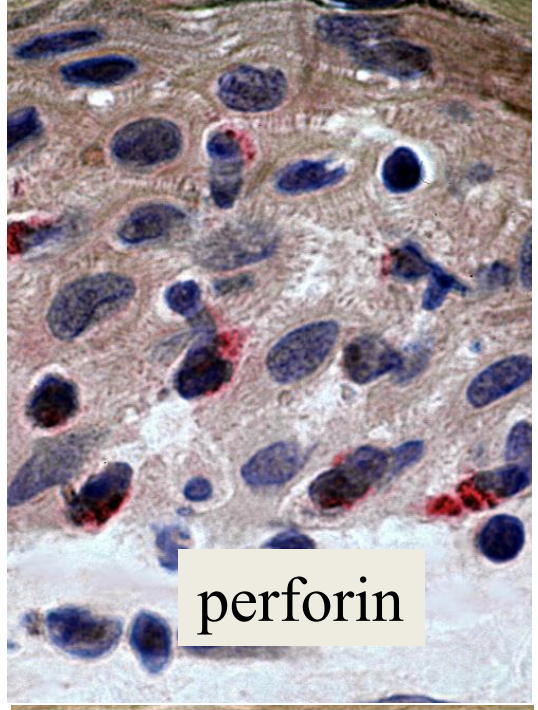
MPE
pustular
bullous

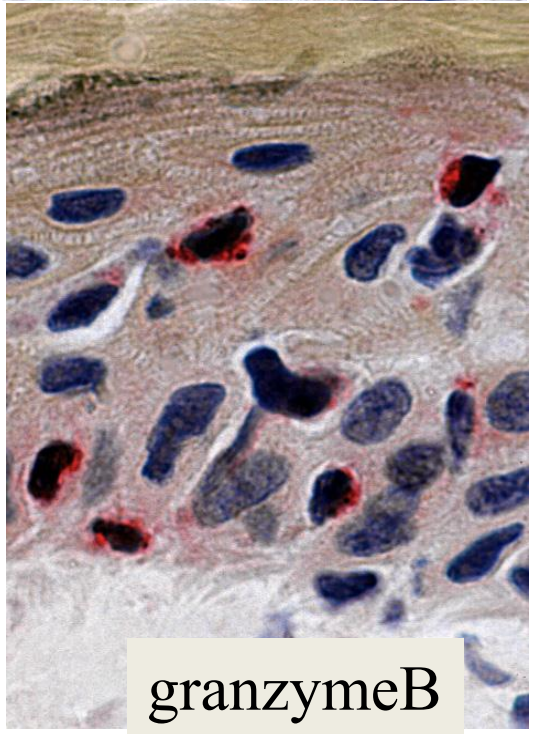


bullous Exanthem:

Perforin+ and GranzymB+
T cells infiltrate epidermis







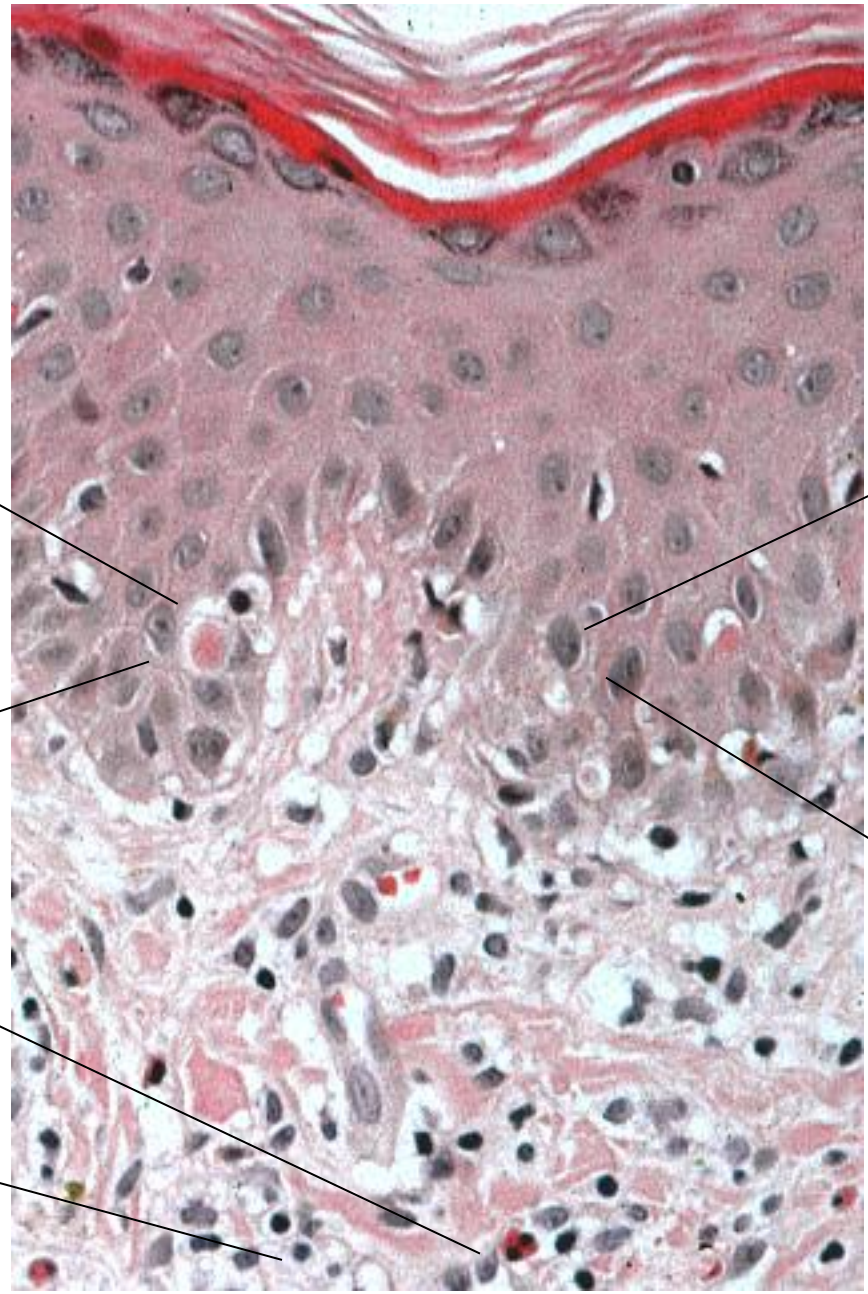
Cytotoxic T cells kill keratinocytes

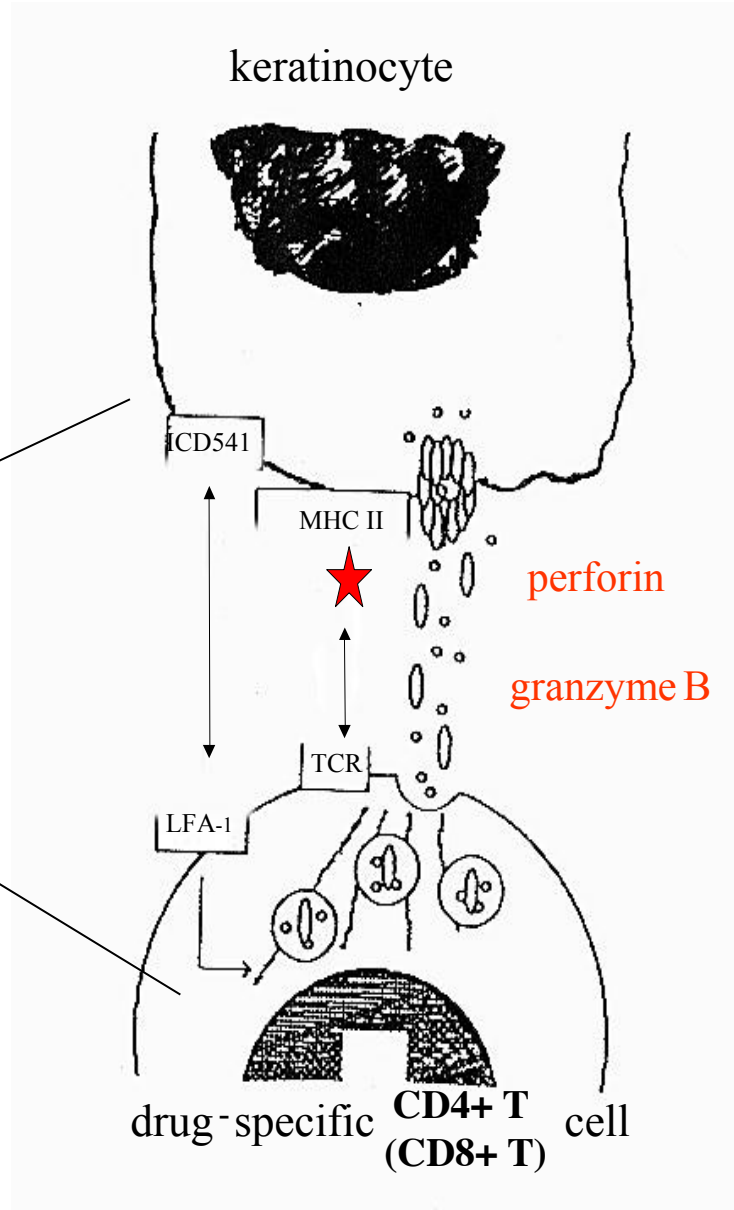
keratinocyte cell necrosis

hydropic degeneration

eosinophils

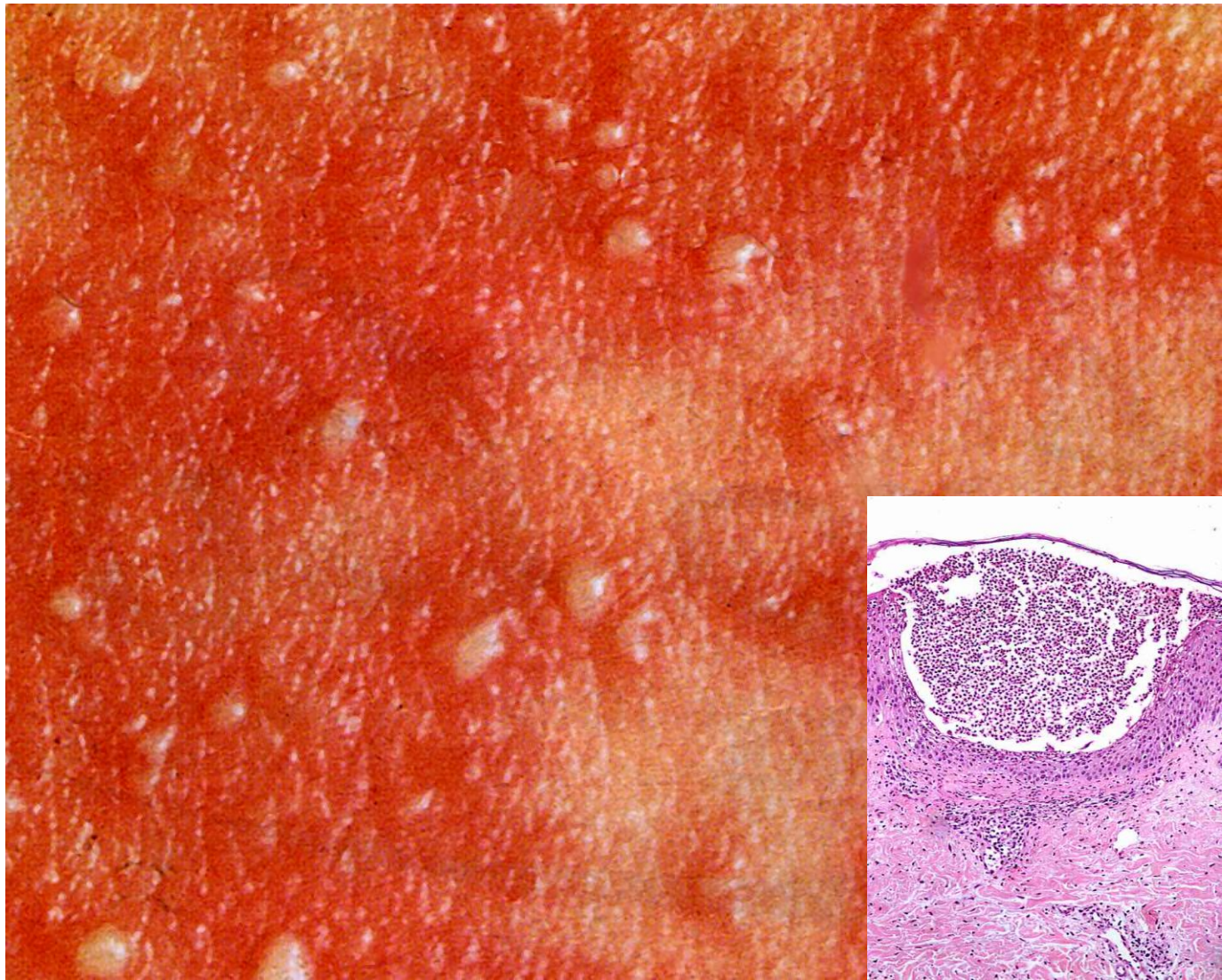
mononuclear cell infiltrate





Acute generalized exanthematous pustulosis

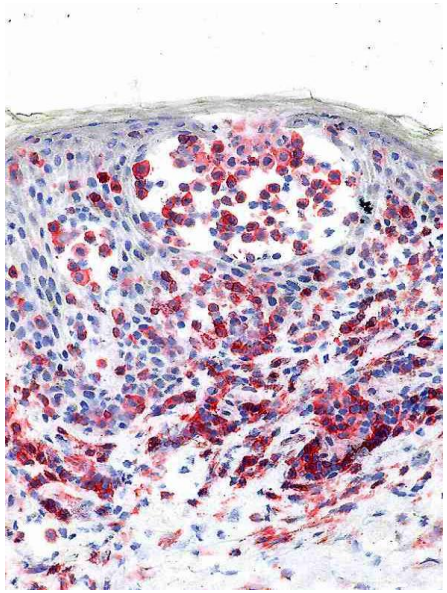
(AGEP)



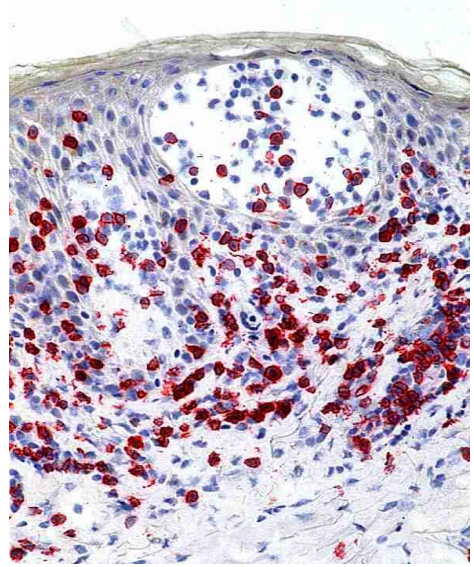
AGEP
a T cell reaction recruiting PMN

FIRST T cells

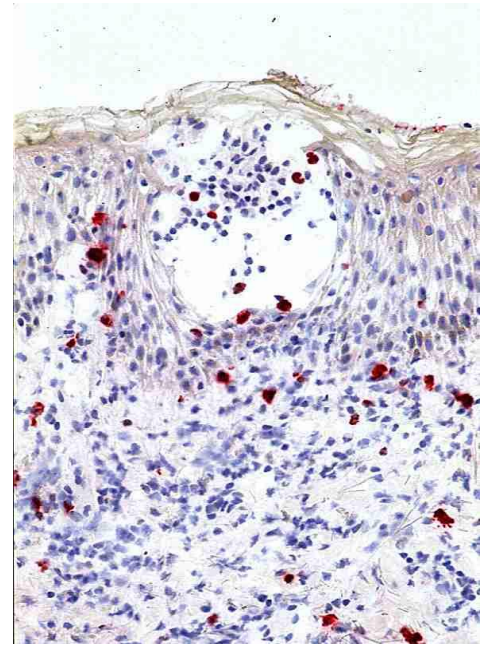
T cell infiltration into epidermis (cytotoxic and IL-8/GM-CSF): vesicle



CD4

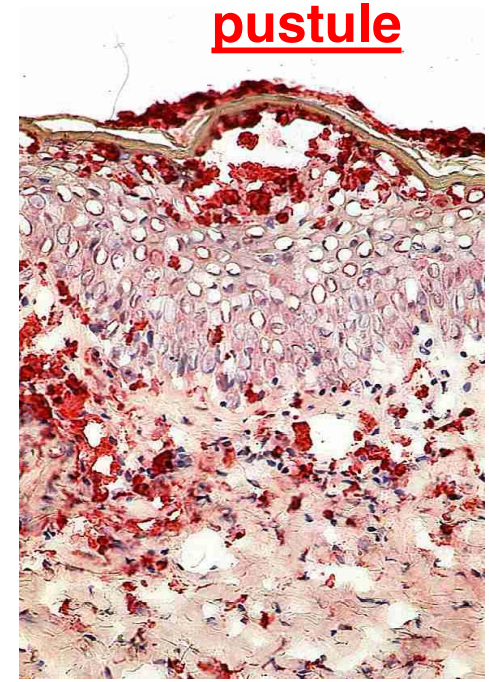


CD8



NEUTROPHIL ELASTASE

SECOND
PMN accumulation
pustule



NEUTROPHIL ELASTASE

later PMN

T-cells react with a drug, are stimulated and expand: they organize a certain pathology

Drug, e.g. amoxicillin

bullous E.

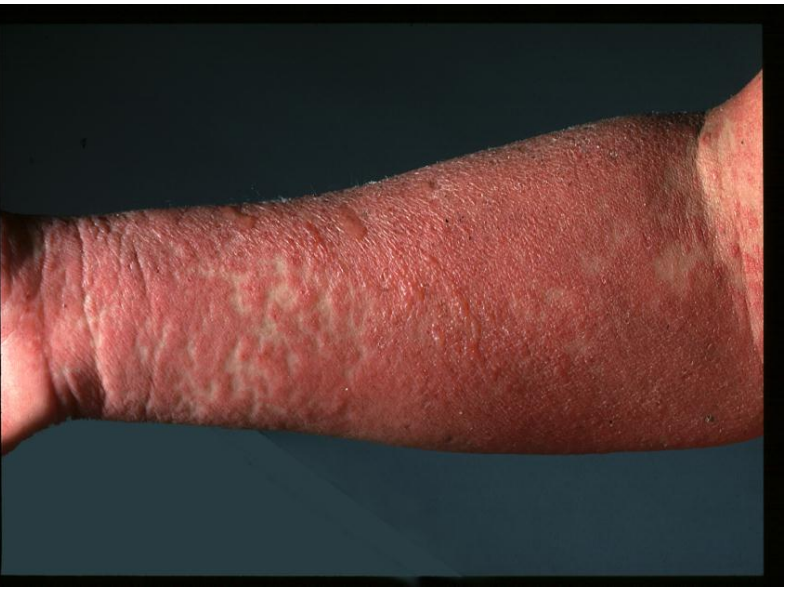
MHC-I (+ MHC-II)
CD8+ > CD4+
cytotoxicity (CD8+)
IFNγ; IL-5

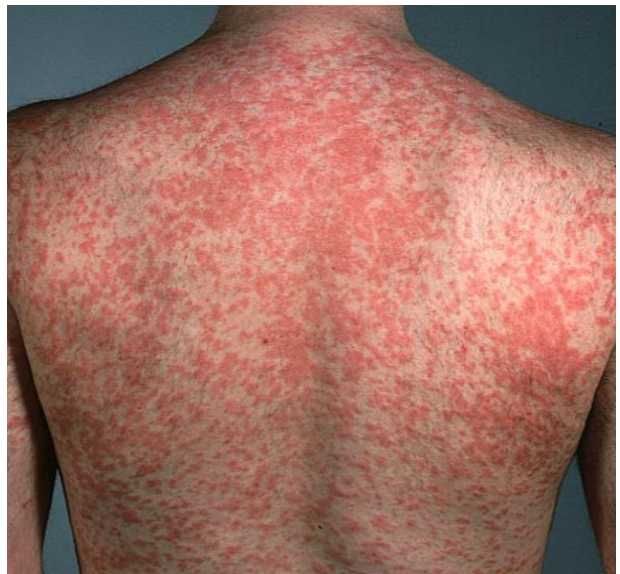
MPE

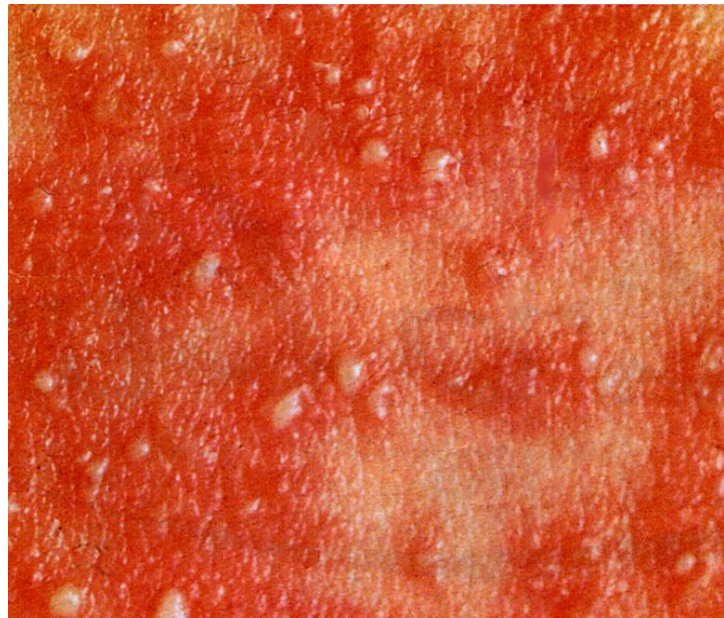
MHC-II
CD4+
cytotoxicity (CD4+)
IL-5; IFNγ

AGEP

MHC-II + I
CD4+ & CD8+
cytotoxicity
IL-8; IL-5

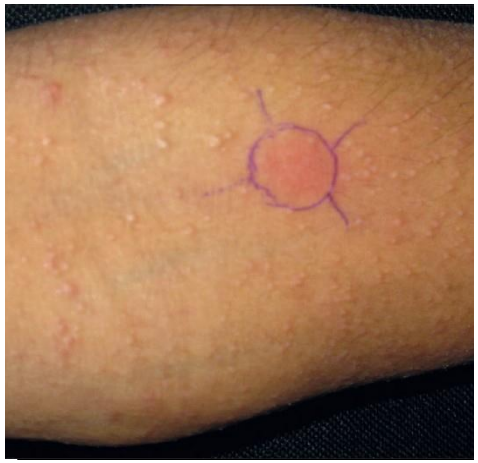


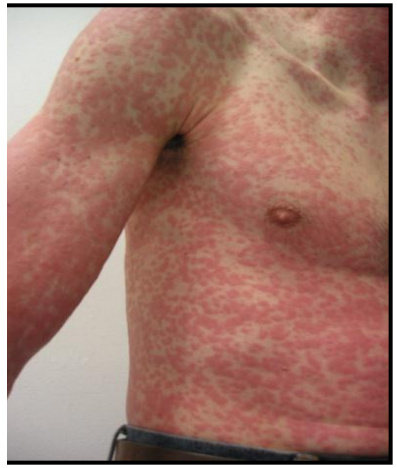


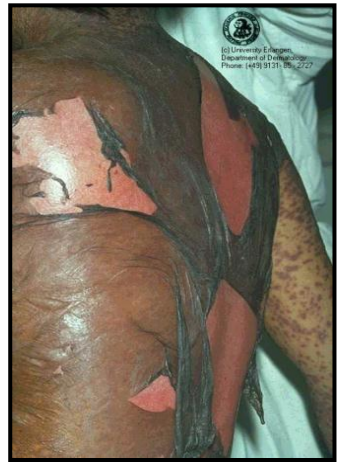


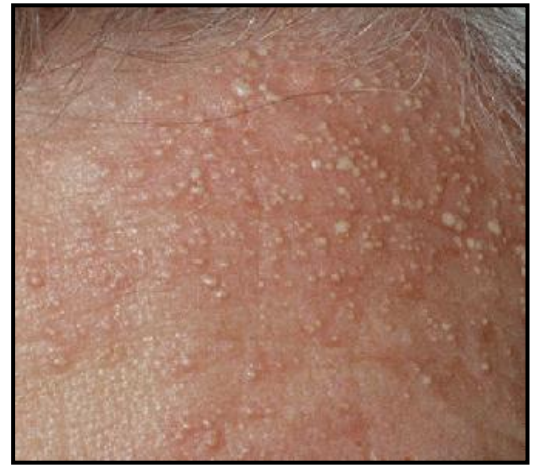
Classification of drug-hypersensitivity reactions

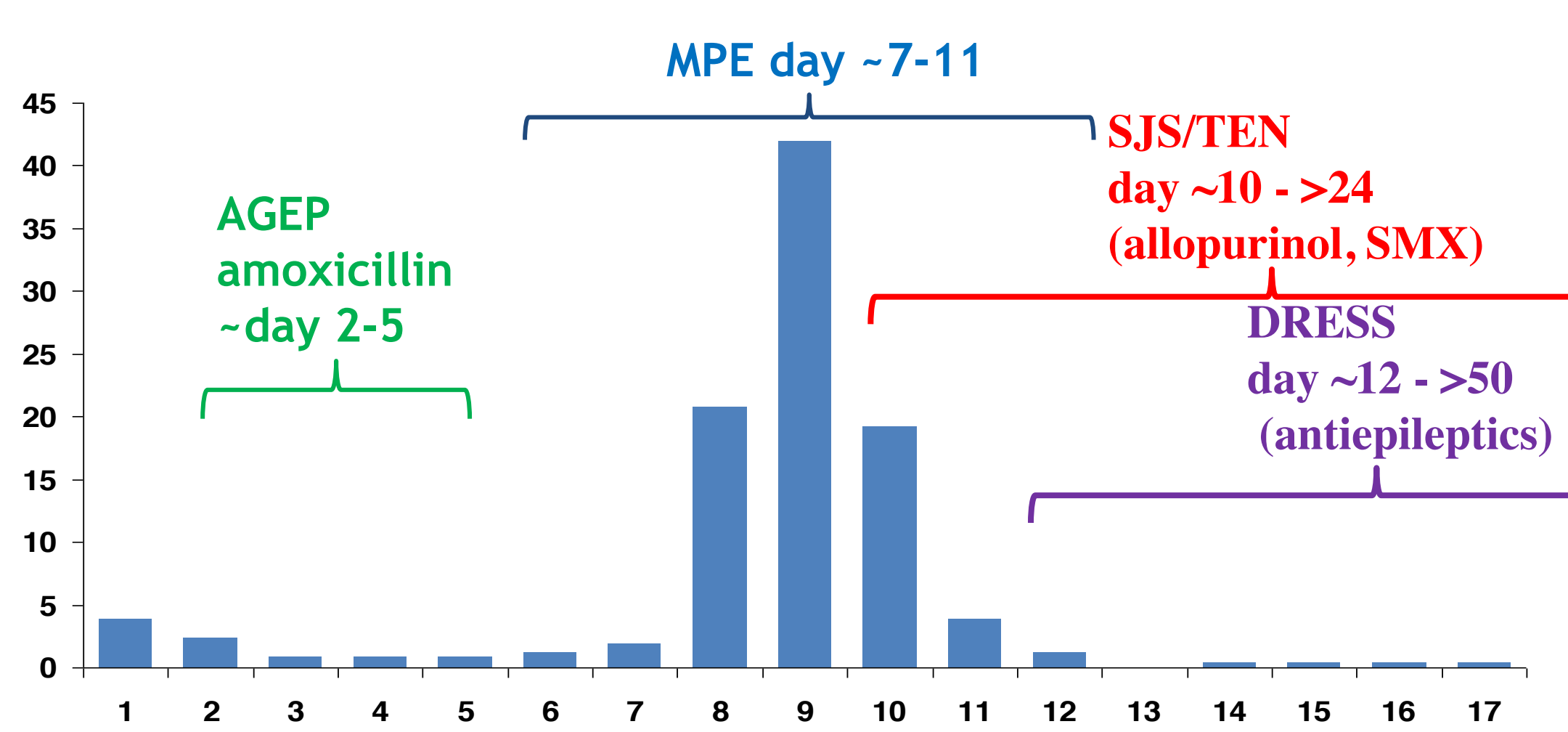
TYPE IV a	TYPE IV b	TYPE IV c	TYPE IV d
Th1	Th2	Cytotoxic T cells	T cells
IFN-γ, TNF-α	IL-5, IL-4, IL-13, eotaxin	Perforin, granzyme B, FasL	CXCL-8, GM-CFS IL-17 (?)
Monocyte, Macrophage	Eosinophilic inflammation	Cytotoxic T cells	Neutrophils
Tuberkulin skin test, (Contact dermatitis)	Maculopapular exanthem with eosinophilia	Contact dermatitis Maculopapular, Bullous exanthema	Pustular exanthema











Time of appearance of delayed skin reactions

Severitiy? Danger signs – delayed reactions

Clinic

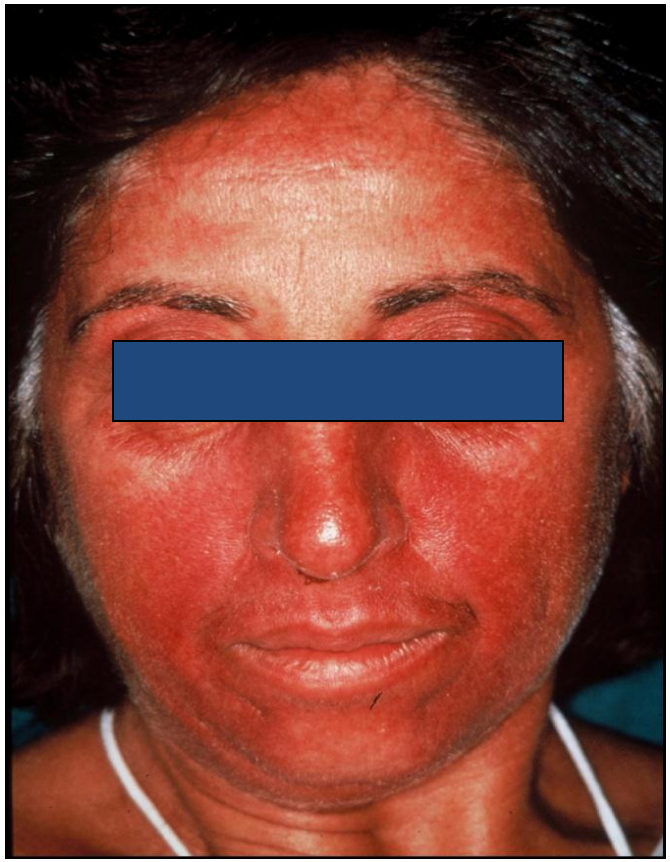
- *widely spread* exanthema
- *induration, bullae, pustules*
- *erythrodermia*
- *pain* in skin
- *Nikolsky* sign
- *mucosal* involvement
- *lymphadenopathy*
- *fever*
- *general symptoms / malaise*
  (liver, kidney, lung, pancreas)

Laboratory

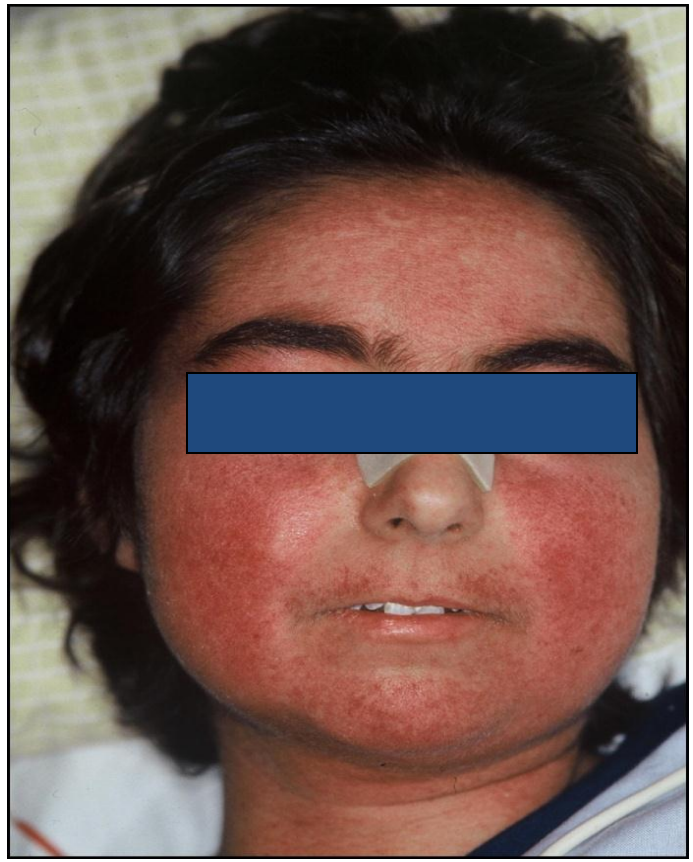
- differential blood count (eosinophilia, activated lymphocytes)
- ALAT, ASAT, γGT, AP
- (CRP ↑~; Creatinine)

delayed reactions: certain laboratory examinations are helpful and necessary

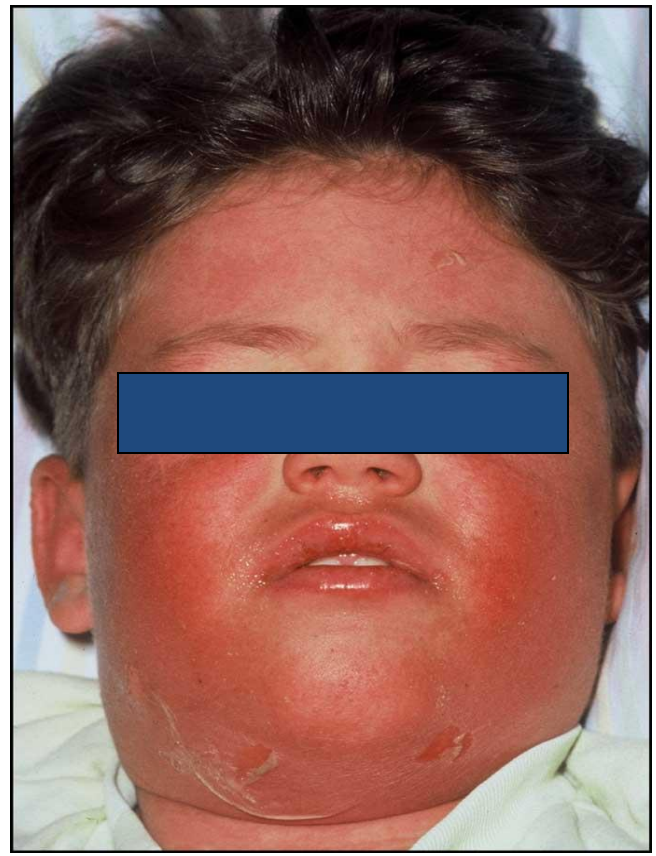
Danger sign: facial edema, flash



DRESS

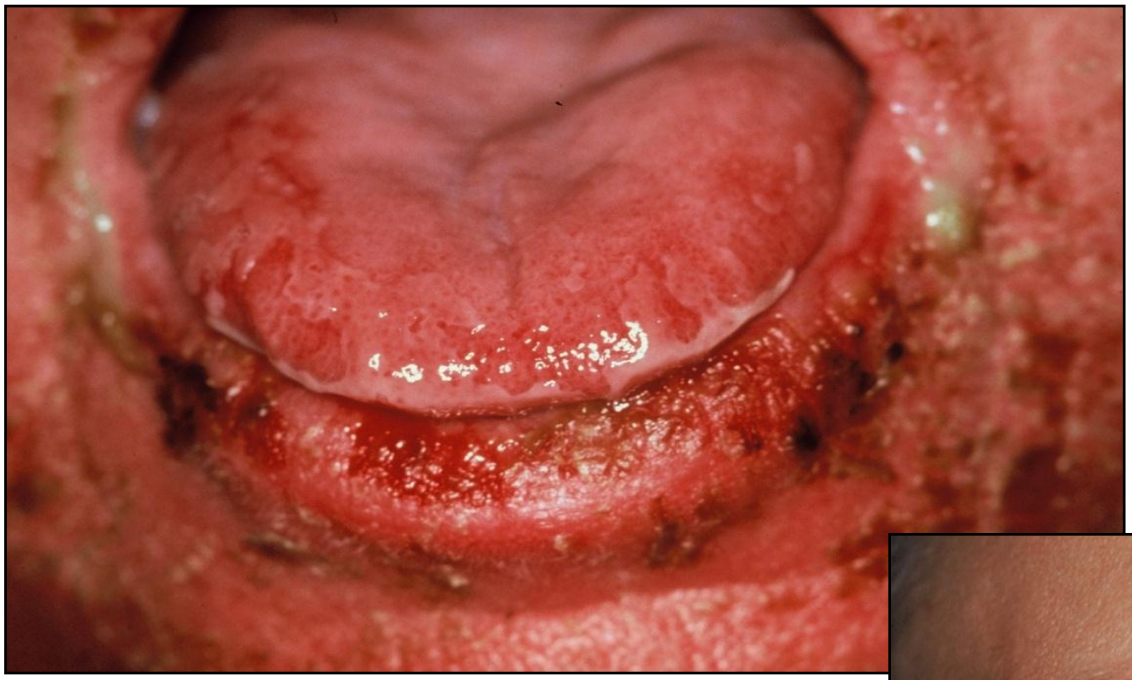


DRESS and haematophagocytic syndrome

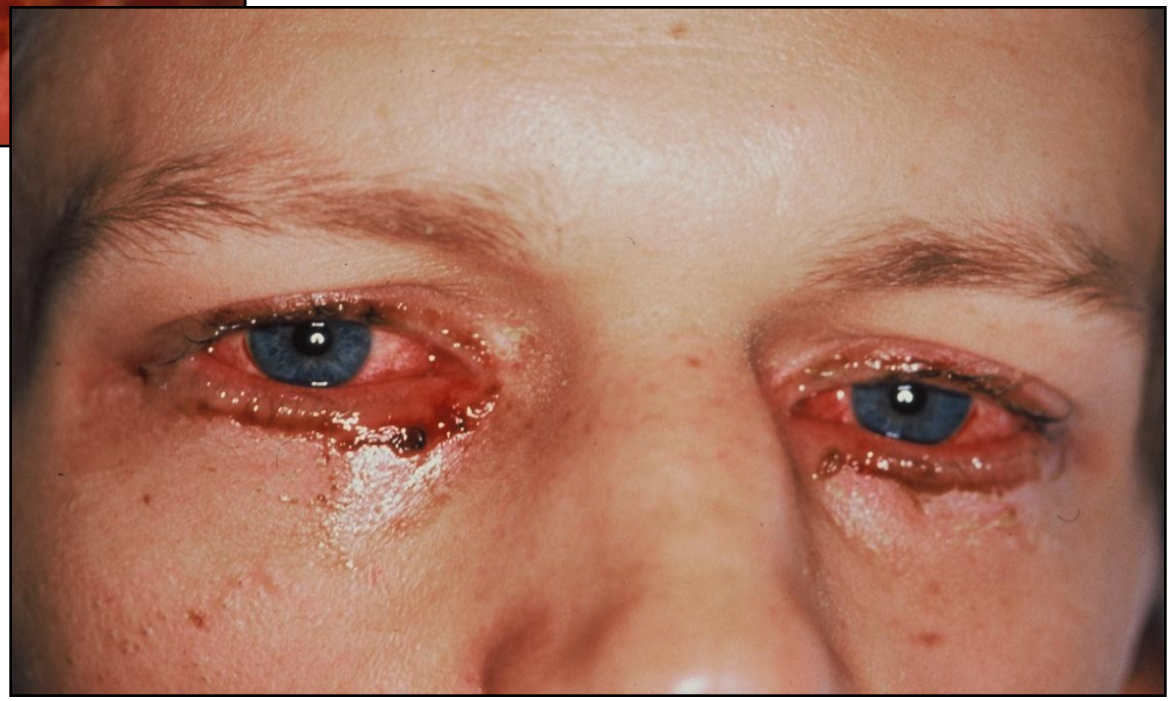


TEN

Danger sign: mucosal involvement



Stomatitis
(SJS, TEN, DRESS)



Conjunctival
involvement
SJS, TEN

Pictures by A Bircher, Basel

Severity? Danger signs – delayed reactions

Clinic

- *widely spread* exanthema
- *induration, bullae, pustules*
- *erythrodermia*
- *pain* in skin
- *Nikolsky* sign
- *mucosal* involvement
- *lymphadenopathy*
- *fever*
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  (liver, kidney, lung, pancreas)

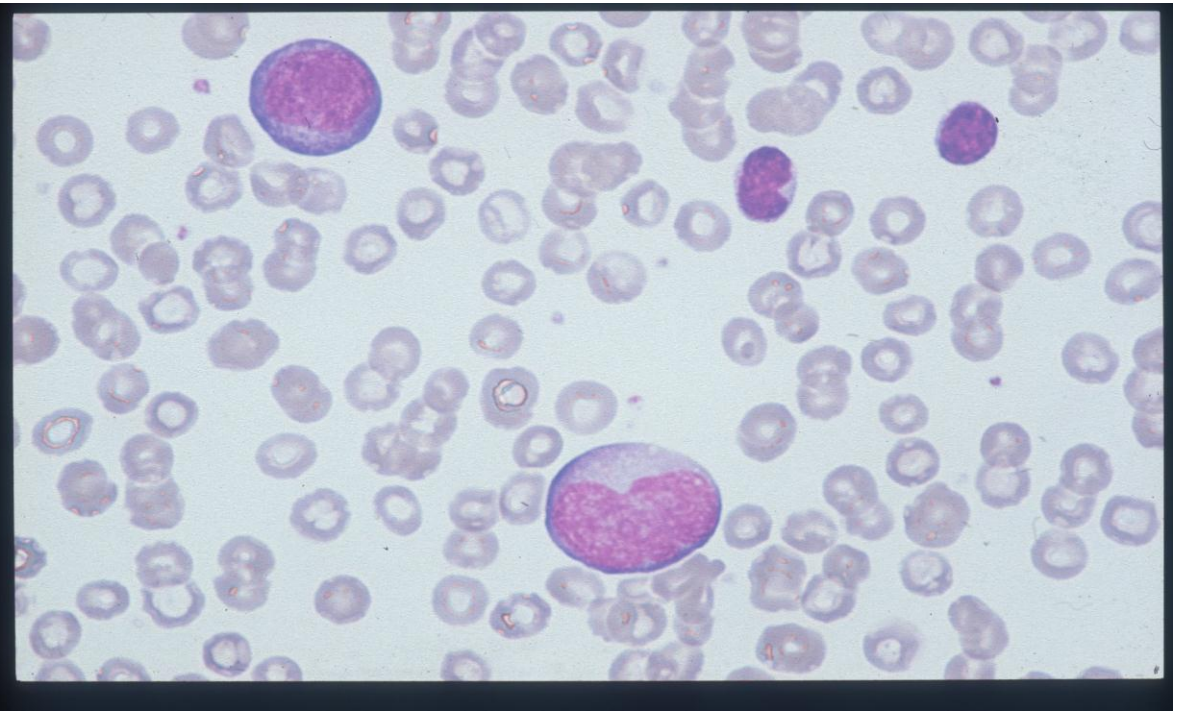
Laboratory

- differential blood count (eosinophilia, activated lymphocytes)
- ALAT, ASAT, γGT, AP
- (CRP ↑~; Creatinine)

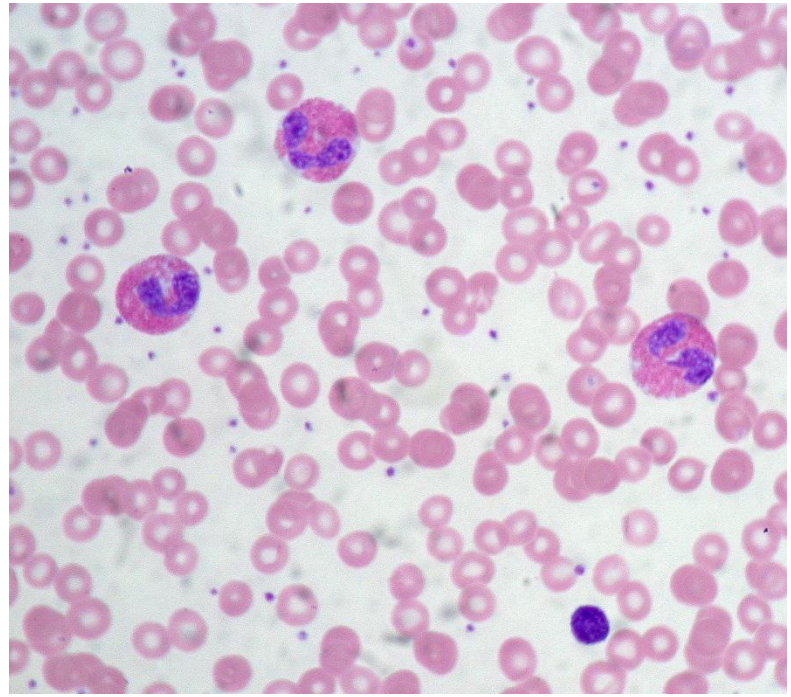
delayed reactions: certain laboratory examinations are helpful and necessary

Danger sign (laboratory): atypical lymphocytes, eosinophilia

„atypical lymphocytes “
(= activated CD8+ T-cells) in the blood at massive immune reactions
(e.g. generalised drug allergy,
acute EBV und HIV-infection, acute Still syndrom...)



eosinophilia (>0,6G/l) is common (~50%) & typical for delayed drug hypersentivity



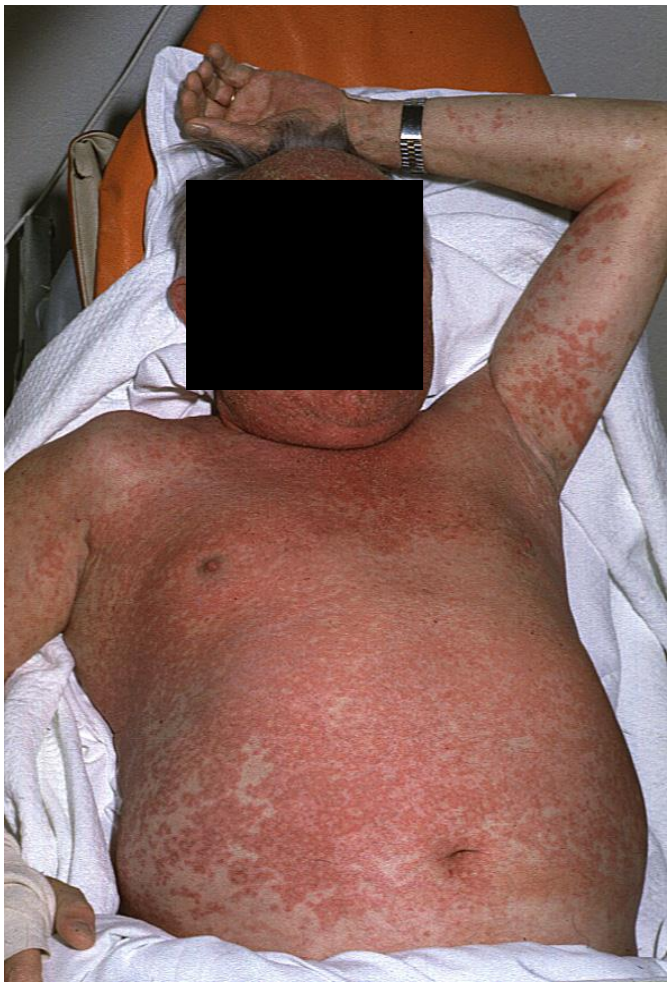
SUMMARY: Exanthematous drug eruptions

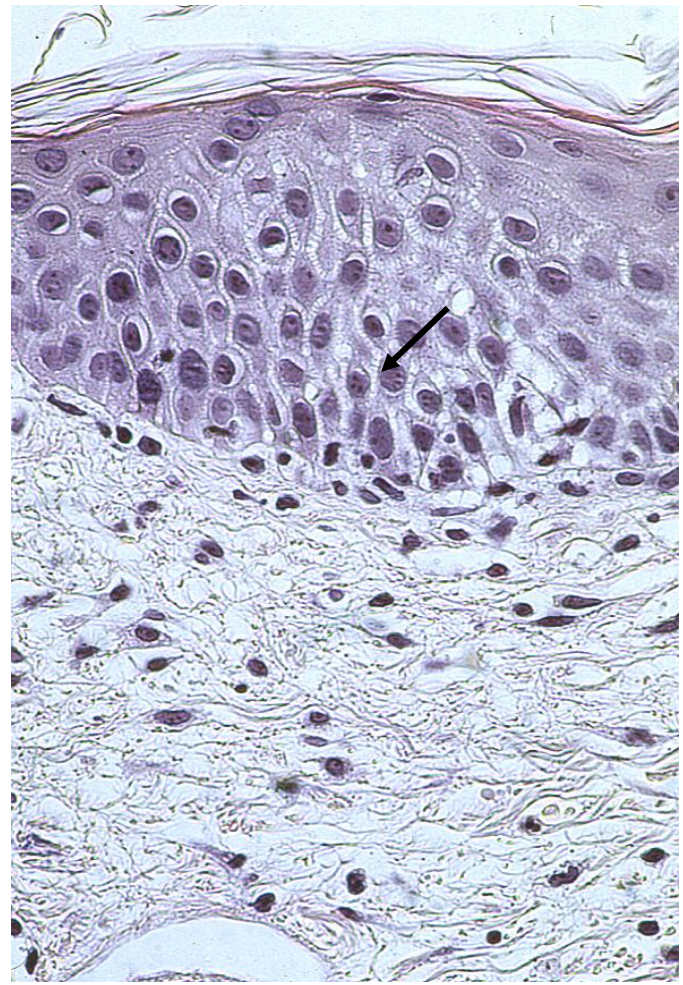
1. Are **T cells** reactions
2. **Timing:** Appear between 2 d (AGEP) and >50 d (DRESS) of drug exposure
3. One **differentiates** papular [MPE], pustular [AGEP], bullous [SJS] (*and macular / urticarial ….*) exanthems
4. determine the **severity** of MPE by clinical and laboratory signs

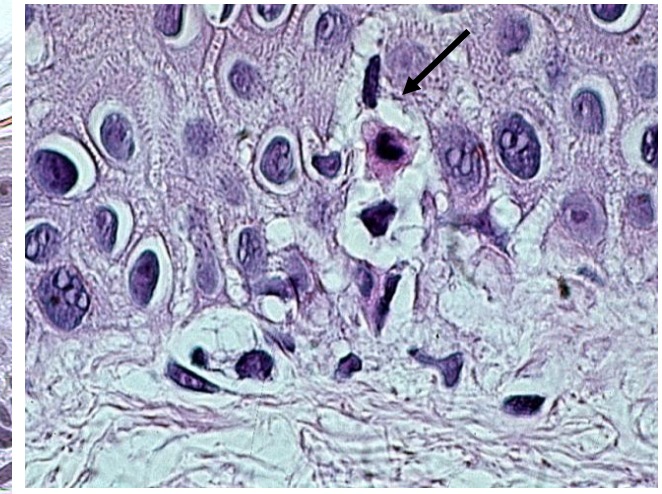
HOW ARE DRUGS STIMULATING T CELLS ?

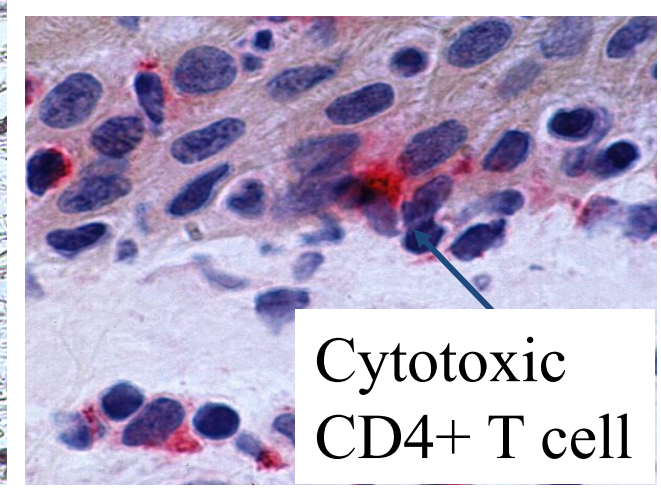
Maculopapular drug eruption (MPE) - Immunohistology

T-cell infiltration into dermis, epidermis;
cytotoxicity (killing of keratinocytes)
recruitment of inflammatory cells

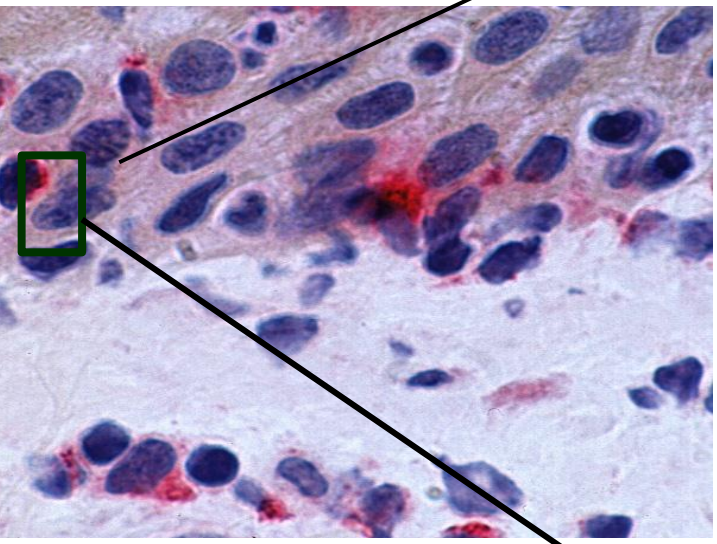




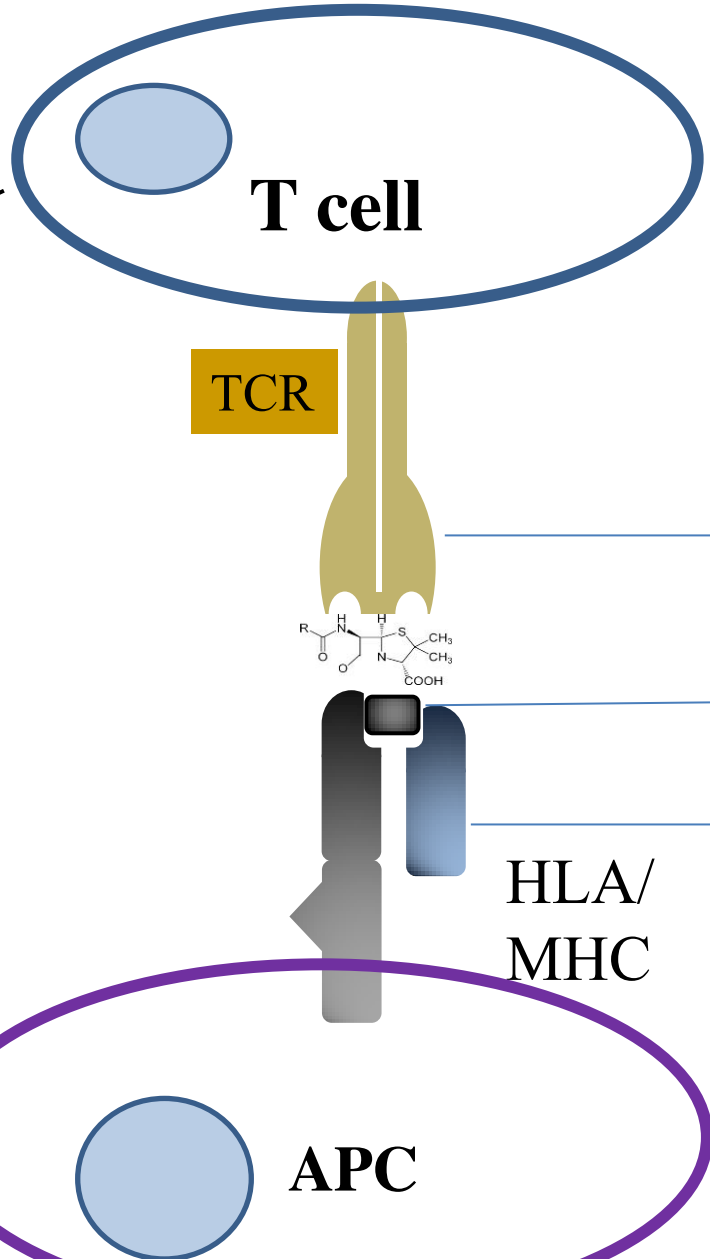


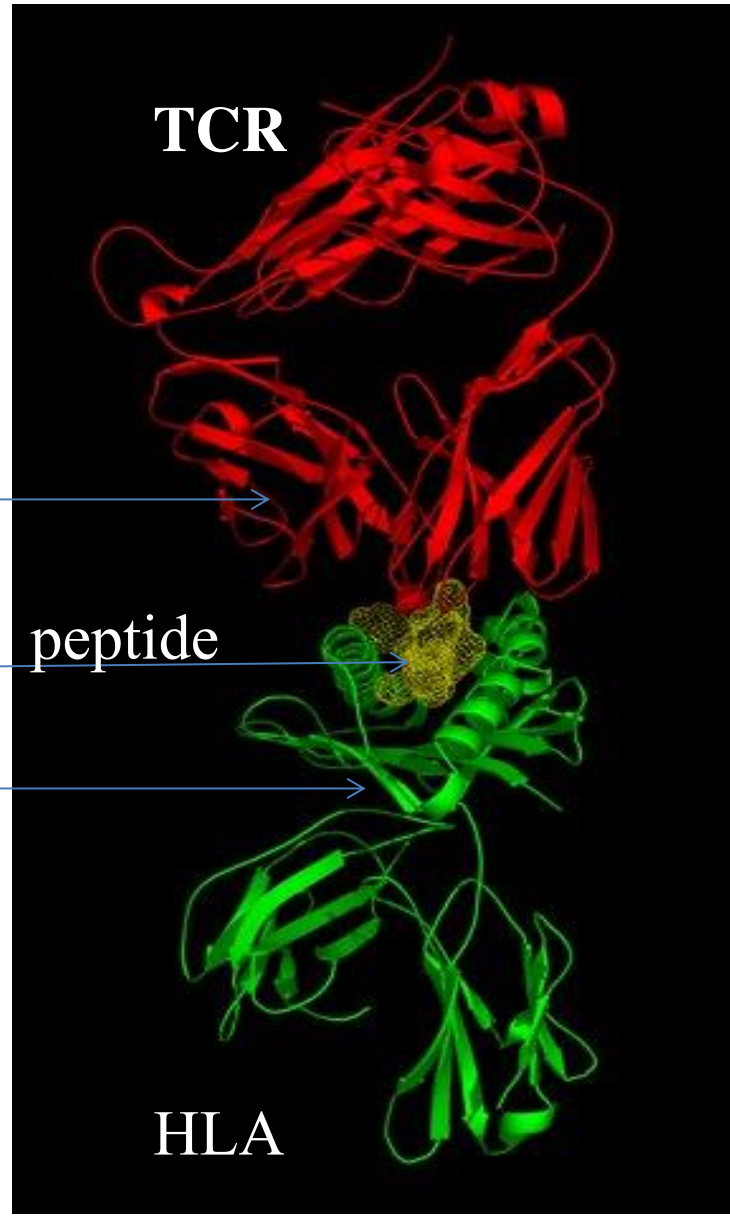


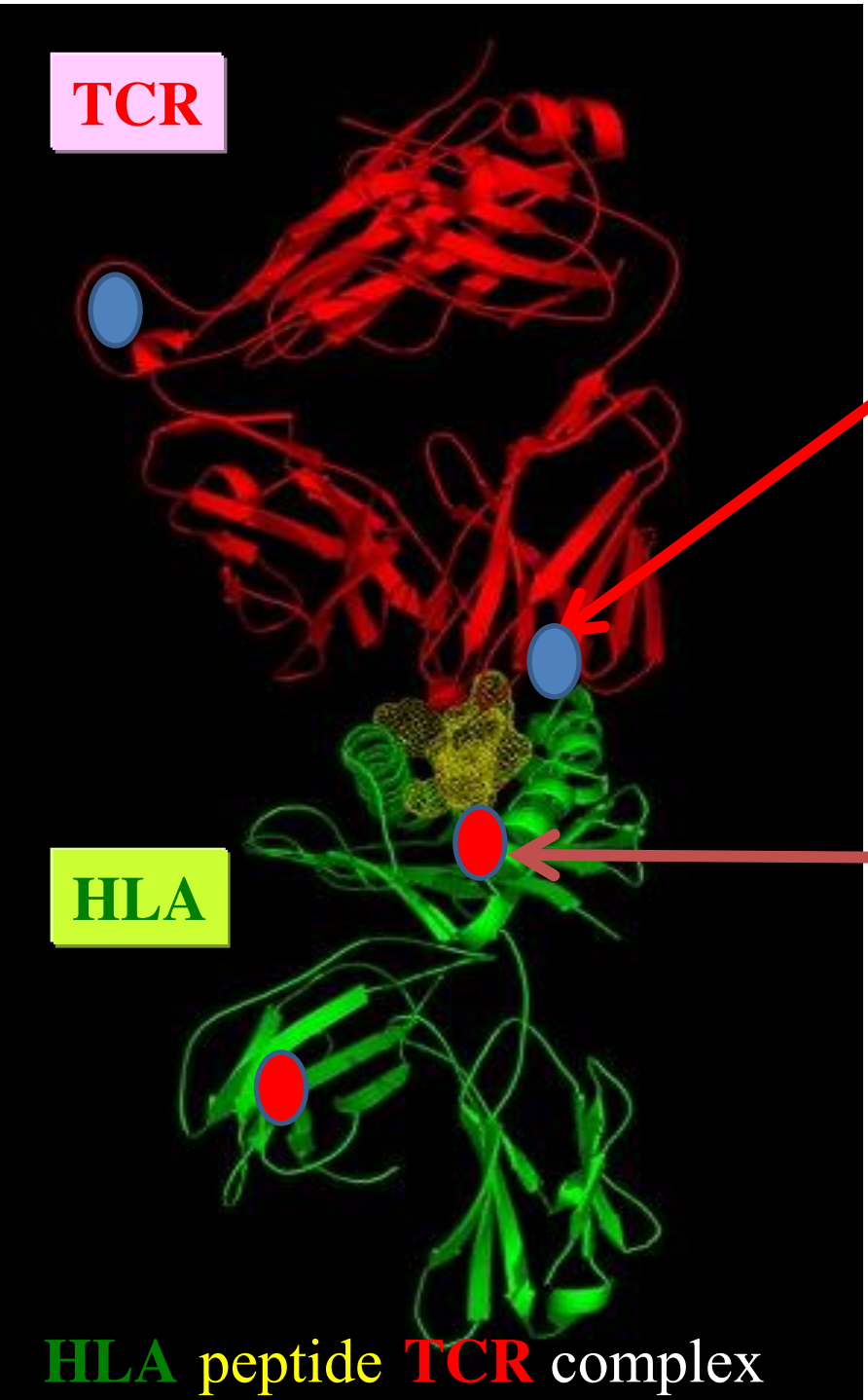
How are drugs stimulating T cells ?



Cytotoxic T cell
killing keratinocytes







pharmacological interaction with immune receptors

(p-i) concept:

a) the drug ● binds to the TCR (by non-covalent bonds; not restricted to a HLA-allele)

or

b) the drug ● binds to the HLA molecule (NOT to the presented peptide);

the {HLA-drug + peptide complex} is recognized by the TCR

Pharmacological interaction with immune receptors = *p-i concept*

It is a non-covalent binding of drugs to proteins functioning as immune receptors (TCR, HLA);

It explains an immune stimulation by a drug without postulating antigen-features of a drug !

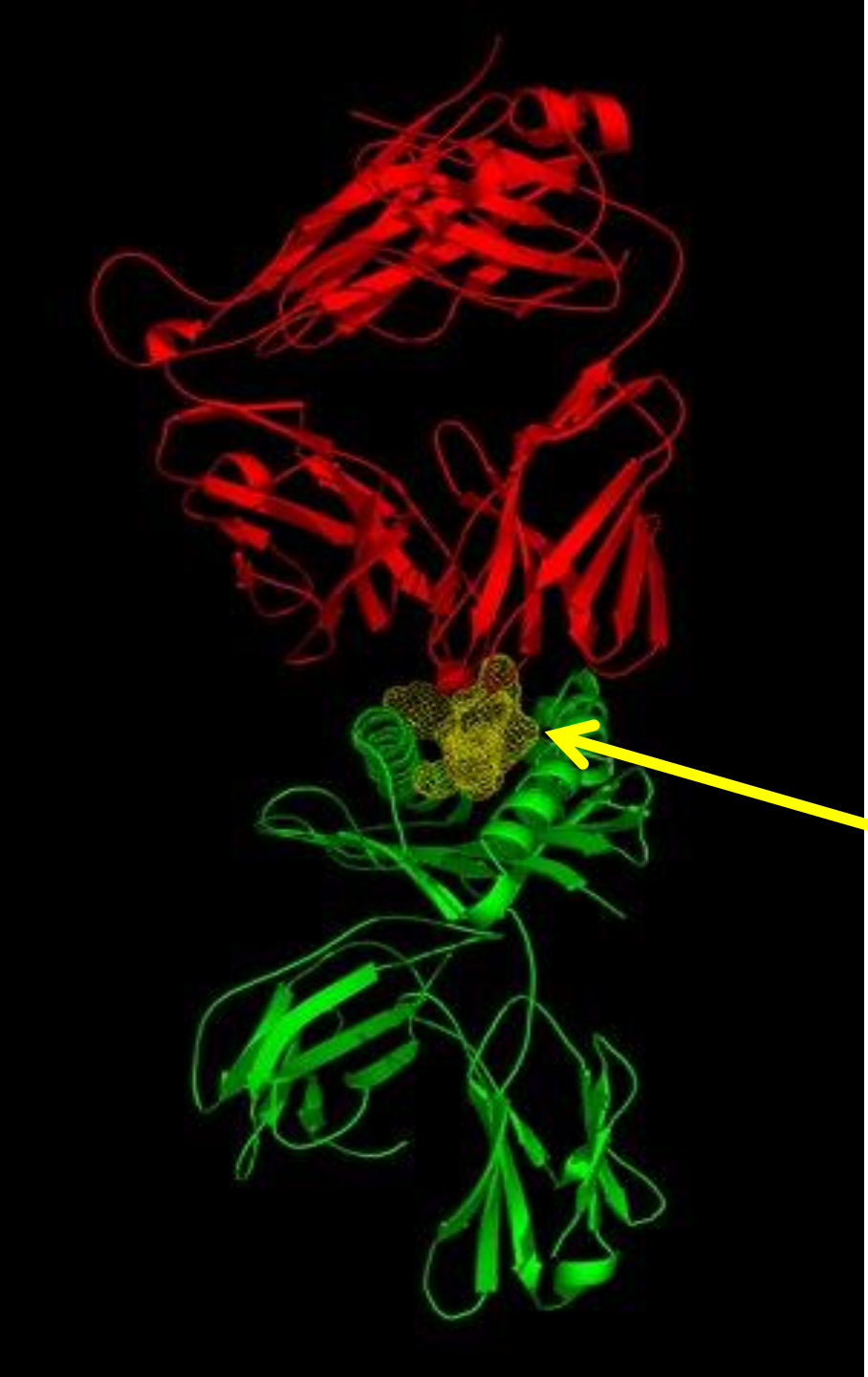
p-i HLA: binding of drug to HLA molecule

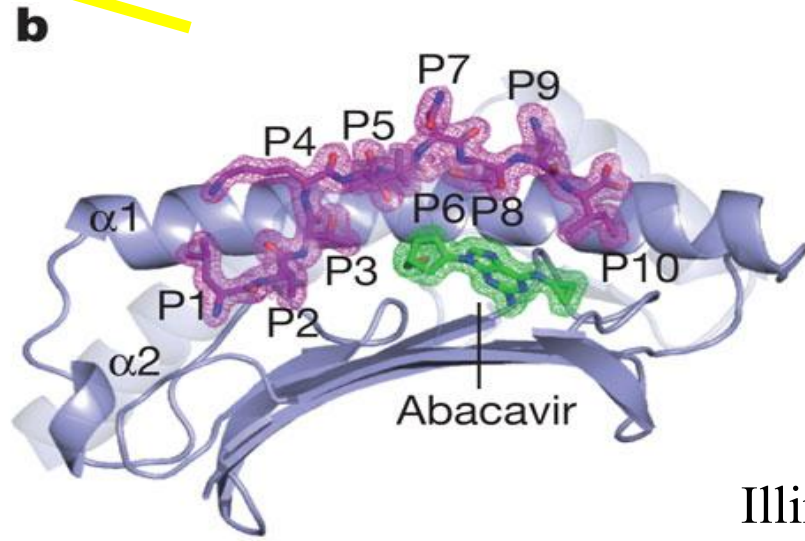
Not the peptide (the «antigen»),

but the HLA molecule itself is modified

p-i concept: a drug fits into a particular HLA molecule

the drug binds to an allele-typic region in the HLA by van der Waals forces;
the {HLA-peptide-drug} complex is then recognized by the TCR

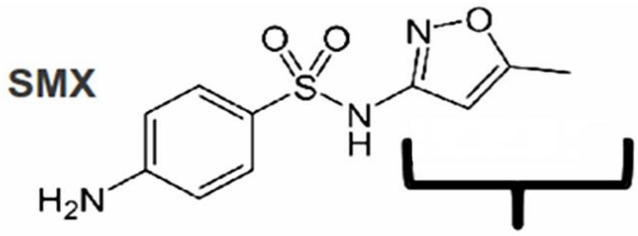


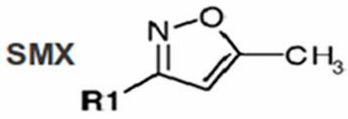


HLA-B*5701: binding groove For abacavir

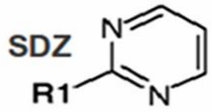
Illing et al, Nature 2012

p-i TCR: binding of drug to T cell receptor (TCR)





Sulfamethoxazole



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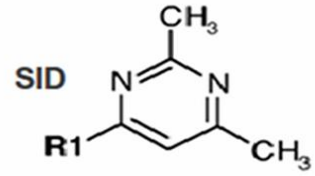

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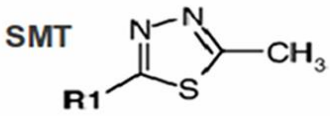

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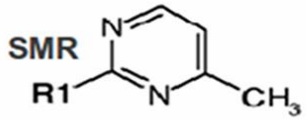

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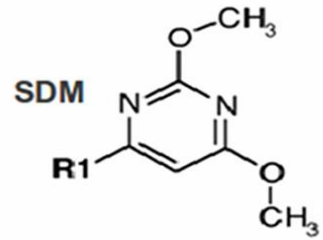
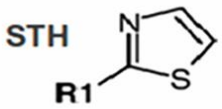


Sulfadimethoxine



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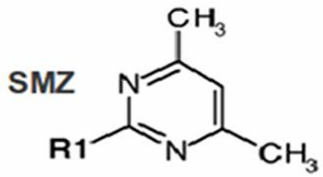
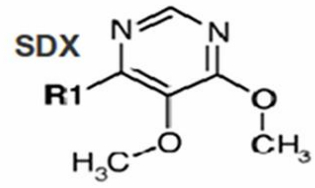
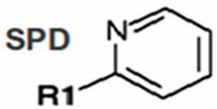


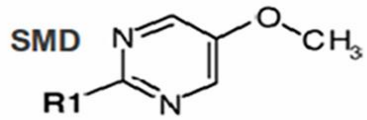
Sulfamethazine



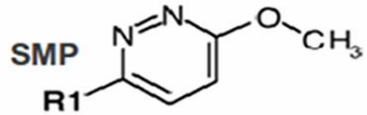
Sulfadoxine



Sulfapyradine



Sulfamethoxidiazine



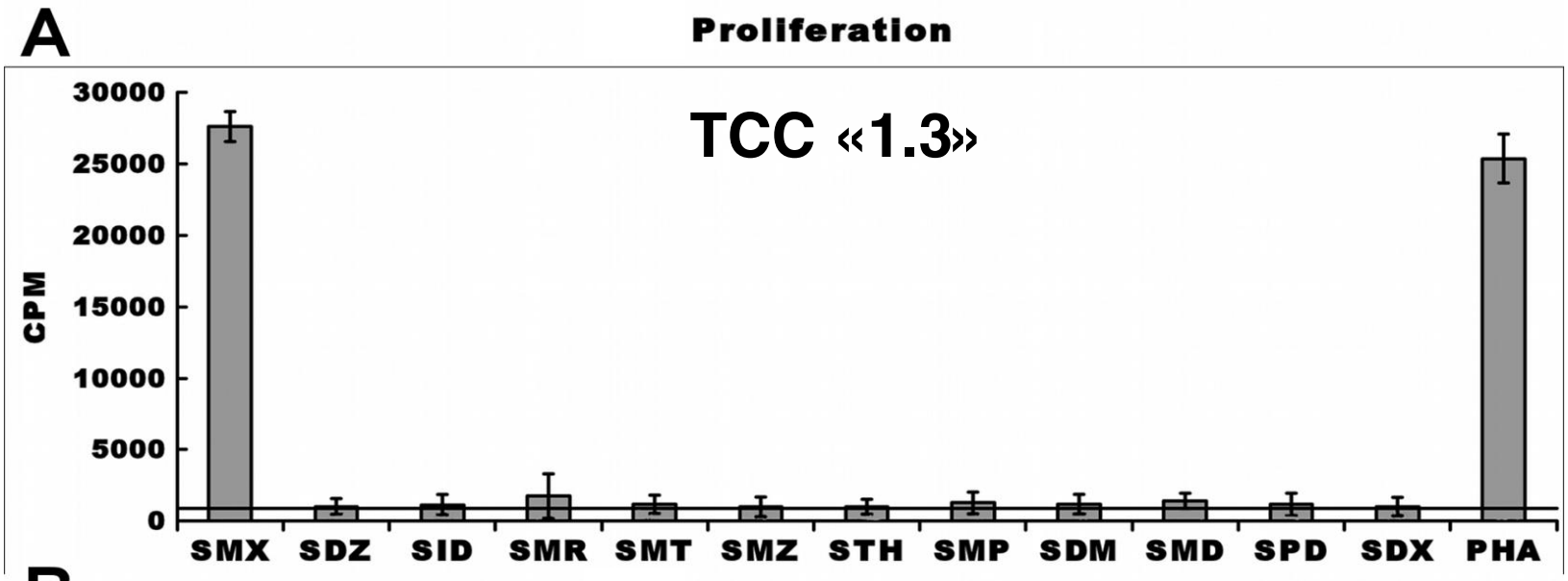
Sulfamethoxipyridazine

p-i TCR:

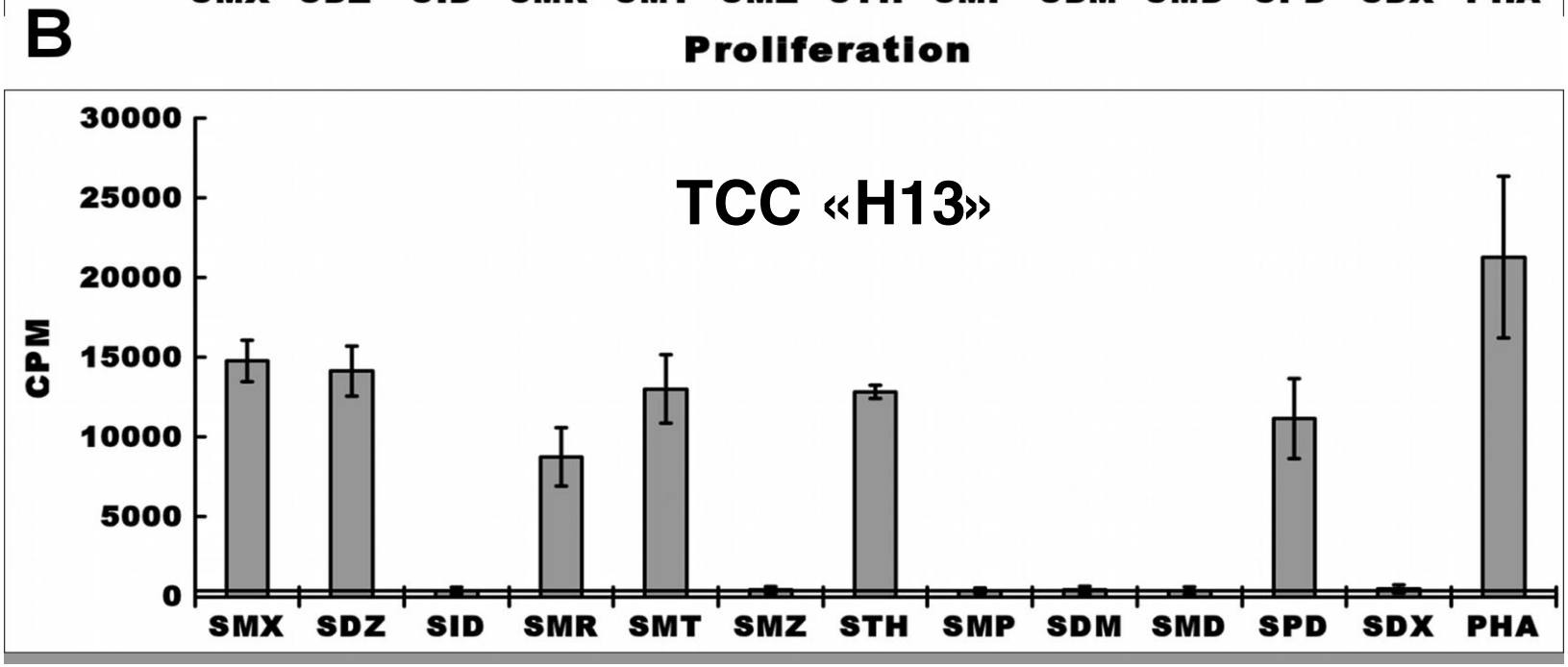
T cell clones specific for sulfamethoxazole (SMX):

- *cross-reactivity*
- *inhibition* of SMX stimulation by other sulfanilamides (n = 11)
- *docking* &
- *dynamic modelling*

Two SMX specific T-cell clones «H13» & «1.3»

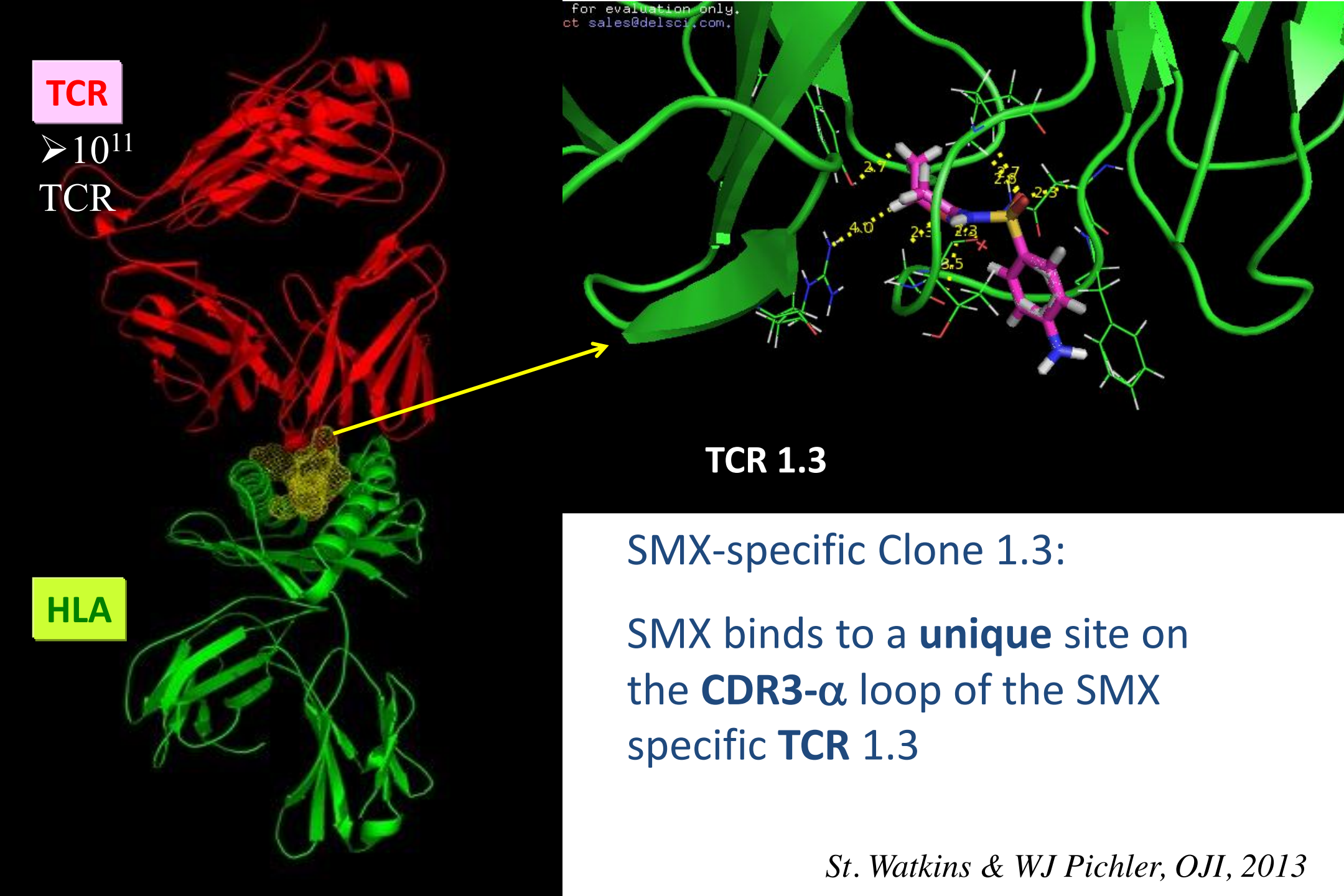


1.3: only SMX;
11 other sulfanilamides (SA) <u>not</u> stimulatory



H13: SMX and 5 other SA stimulatory

St Watkins & WJ Pichler, OJI, 2013

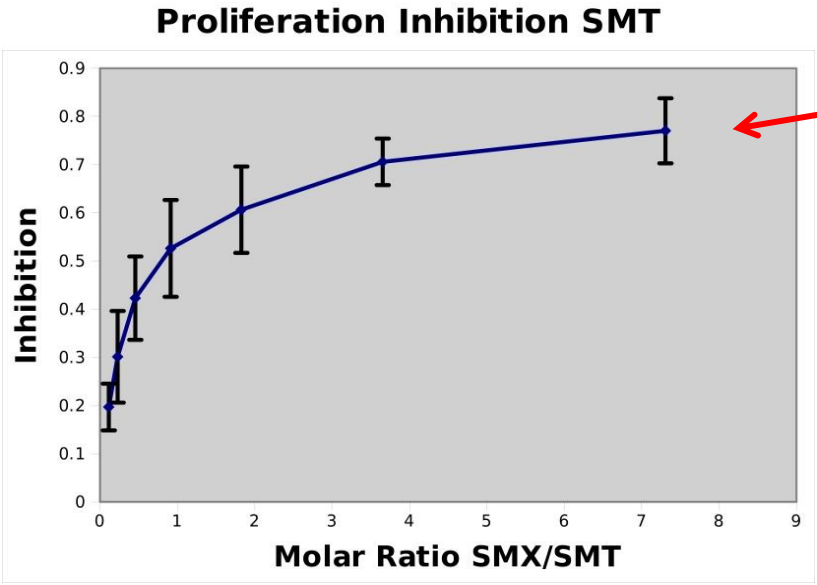


SMX-specific Clone 1.3:

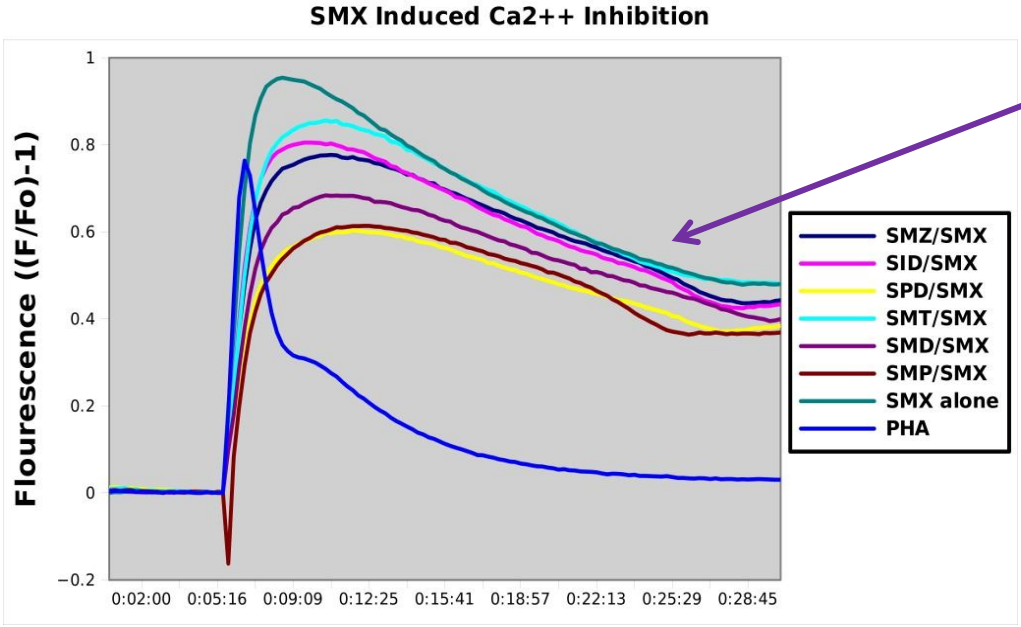
SMX binds to a **unique** site on the **CDR3-α** loop of the SMX specific **TCR** 1.3

St. Watkins & WJ Pichler, OJI, 2013

TCC 1.3



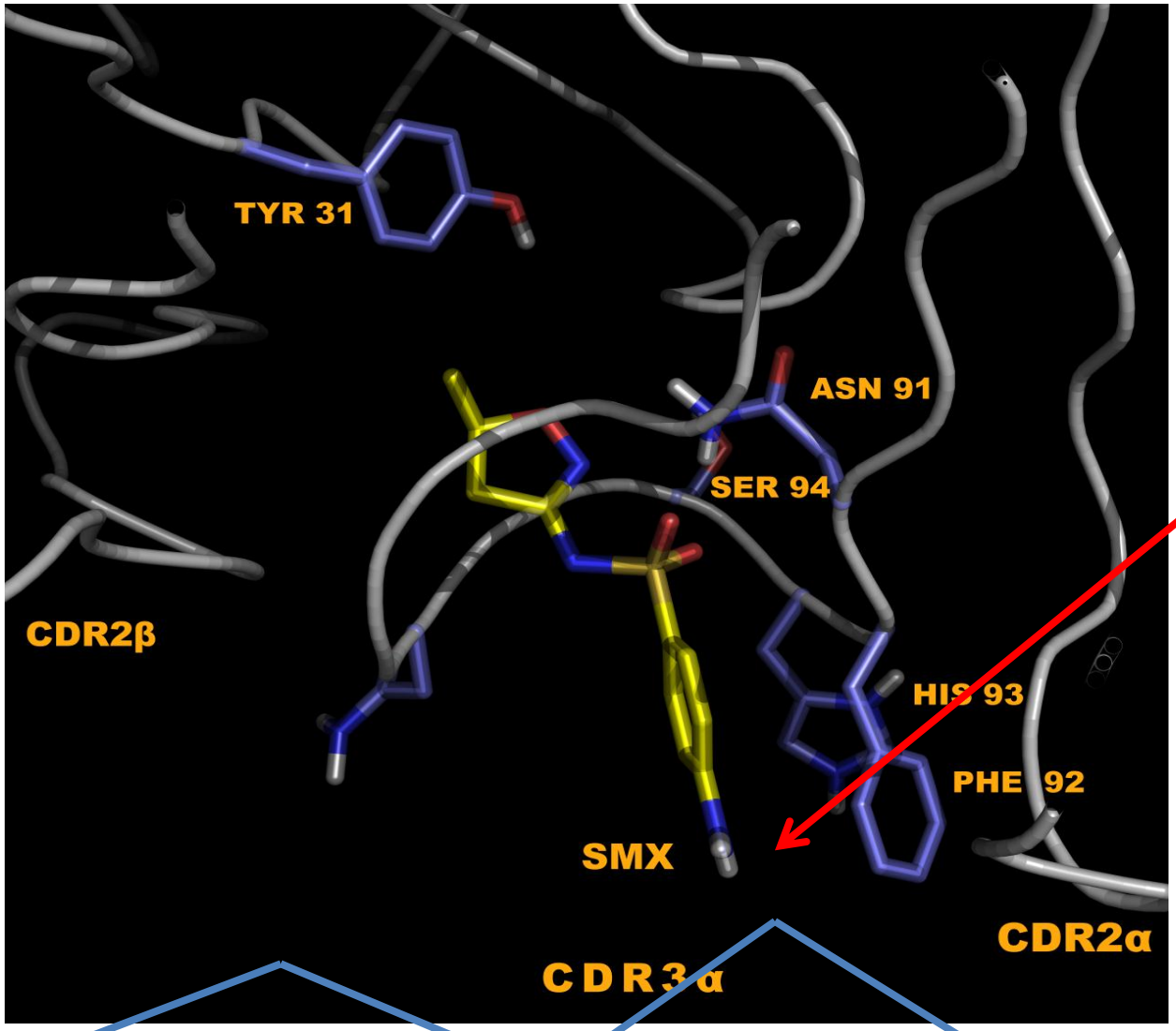
75% inhibition of SMX induced proliferation by the sulfanilamide SMT (sulfamethazole)



35% inhibition of SMX induced Ca++ influx by sulfanilamides

St Watkins & WJ Pichler, OJI, 2013

Clone 1.3



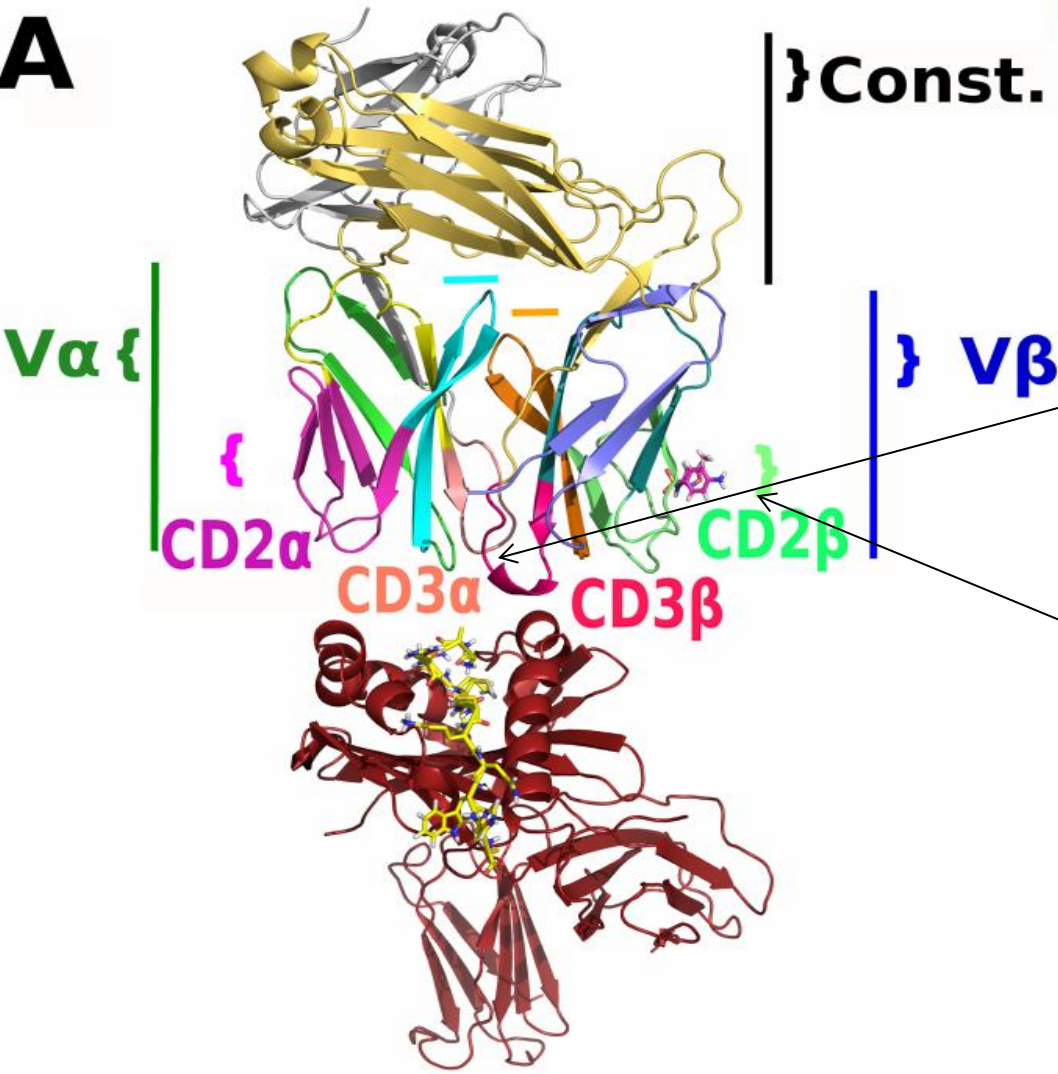
- The TCR 1.3 showed CDR3α recognition of SMX.

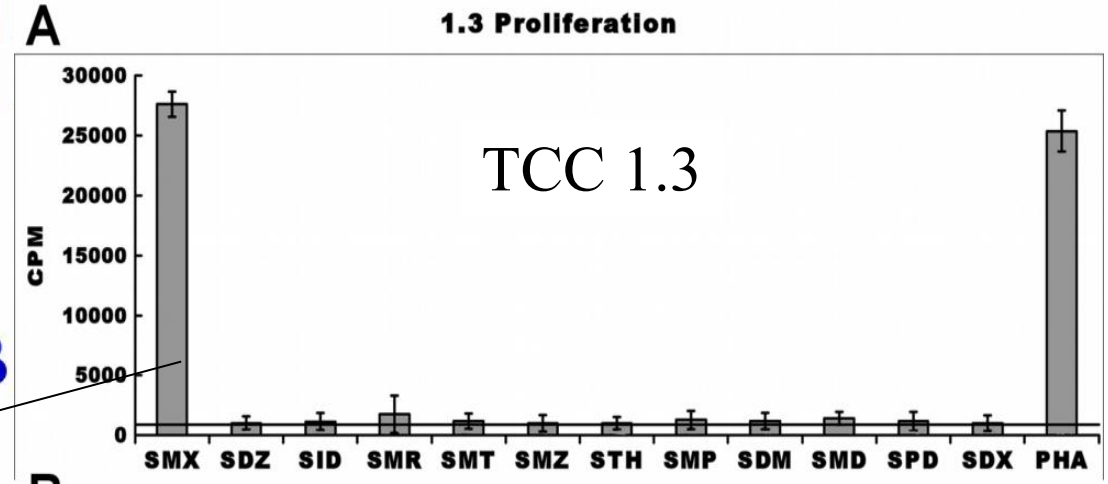
The NH2 of SMX may contact the peptide.

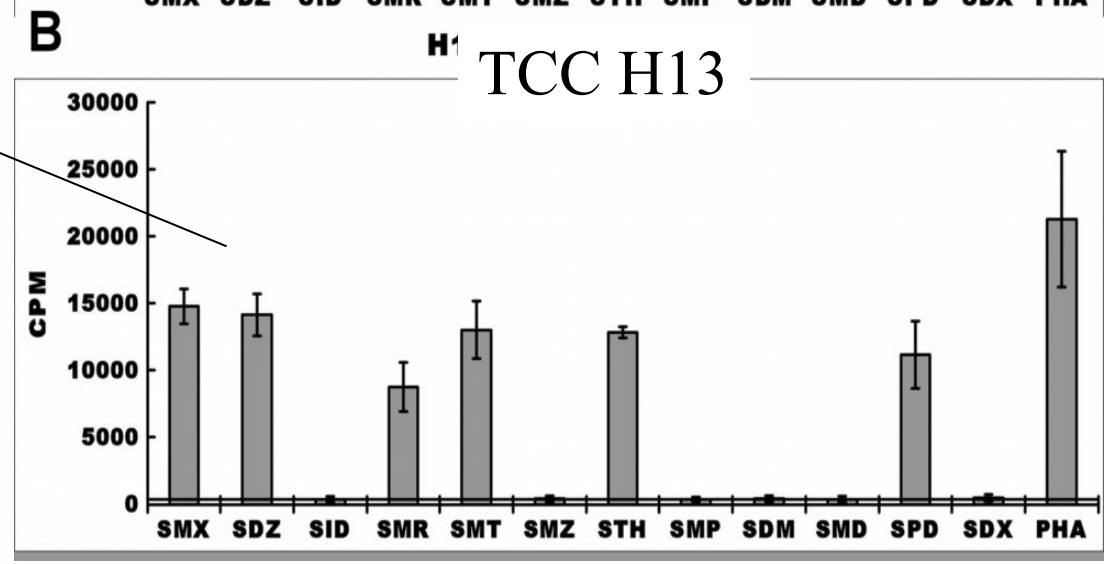
This may explain the cross-reactivity of some TCC reactive with hapten (SMX-NO) and via p-i (SMX)*

*Schnyder B et al. J Immunol. 2000

SMX is bound to TCRVβ2 of TCR «H13», outside the HLA-peptide interaction site!

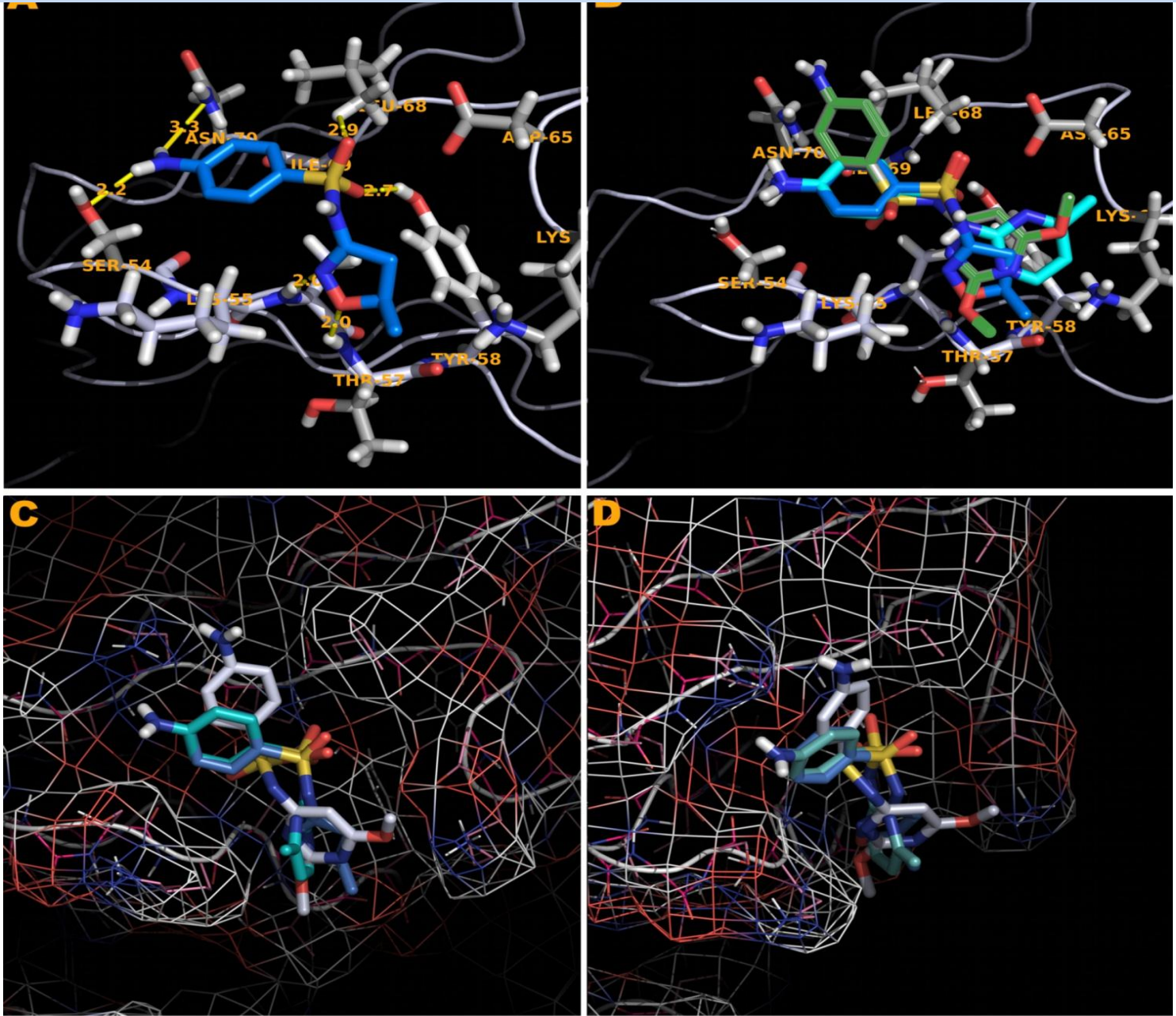






St Watkins & WJ Pichler: Sulfamethoxazole Induces a Switch Mechanism in T-Cell Receptors Containing TCRVβ20-1, by Altering pHLA Recognition; PLOS One, 2013

Clone H 13

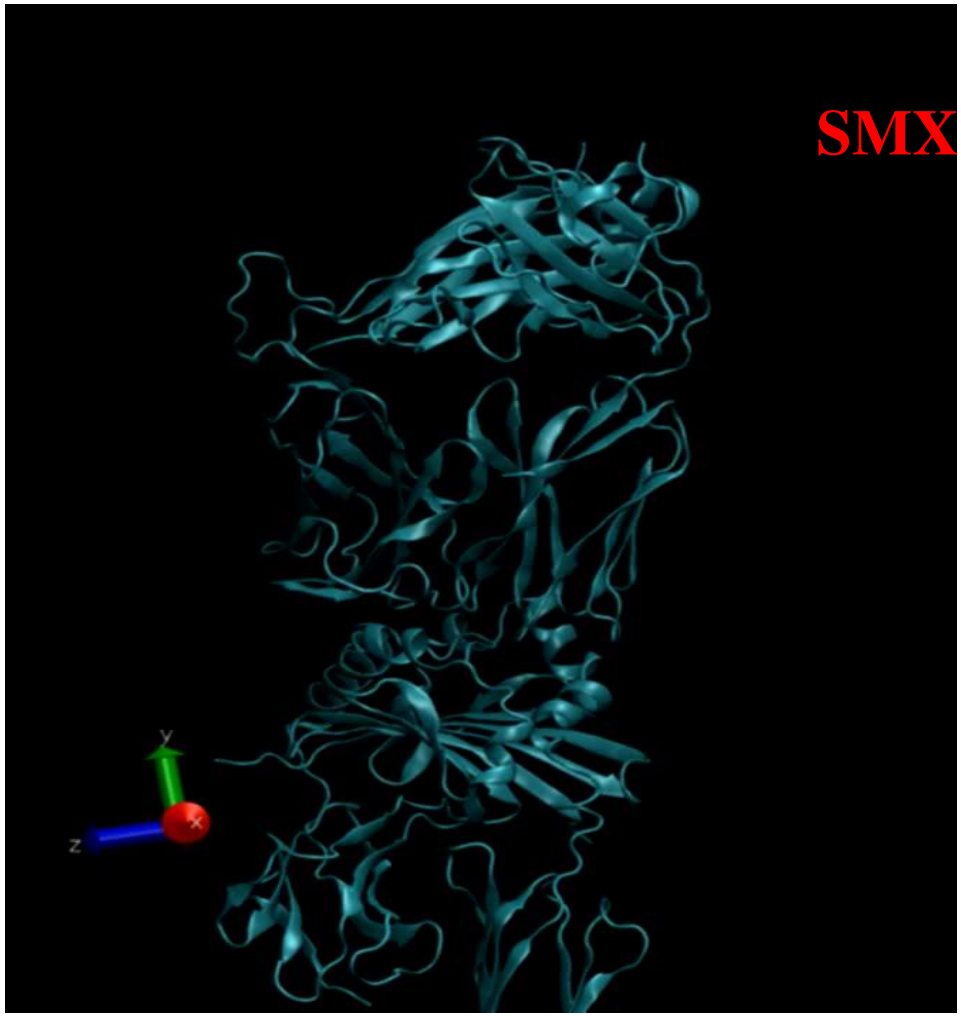


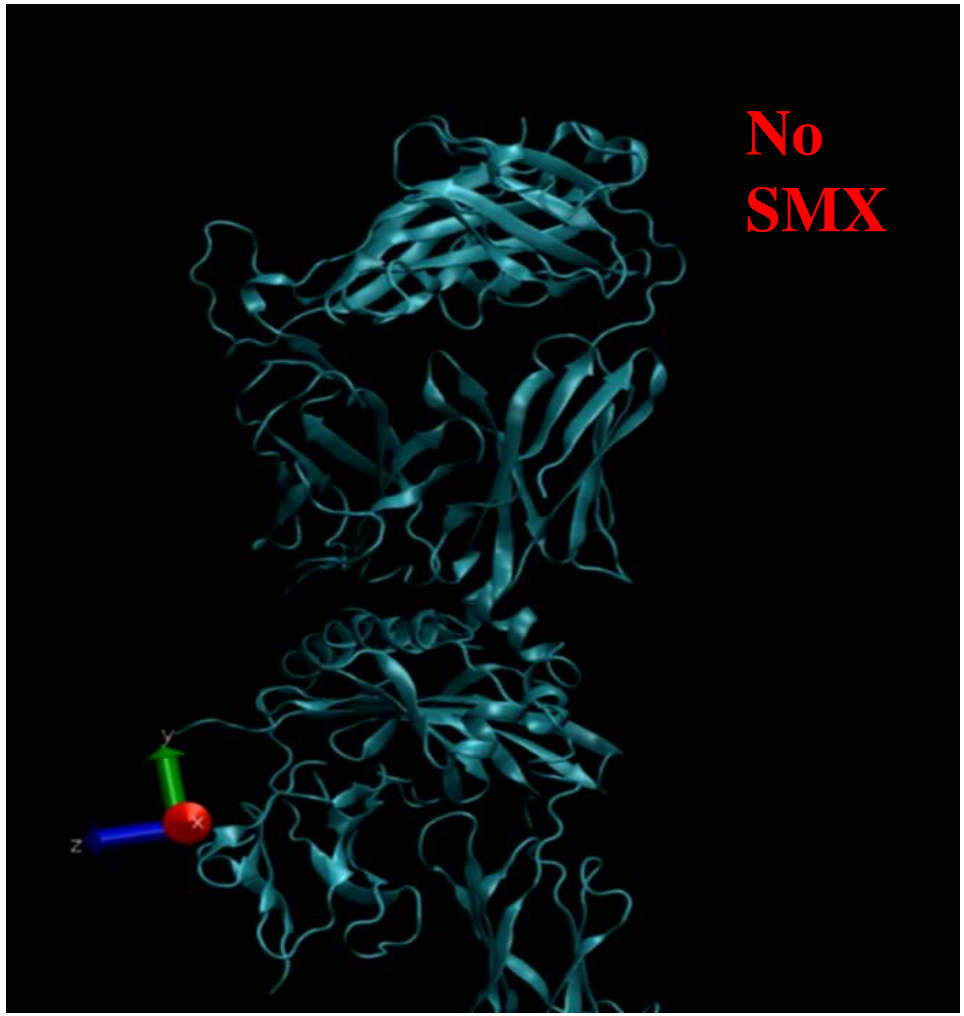
Drug (SMX) binding to the TCR-Vβ CDR2 loop.

Only SMX and 5 of 11 other sulfanilamides fit into the pocket formed by the CDR2 region (TCR H13)

Stephan Watkins & Werner J. Pichler: Activating Interactions of Sulfanilamides with T Cell Receptors, Open J Immunology, 2013

Visualizing the H13 Binding Process

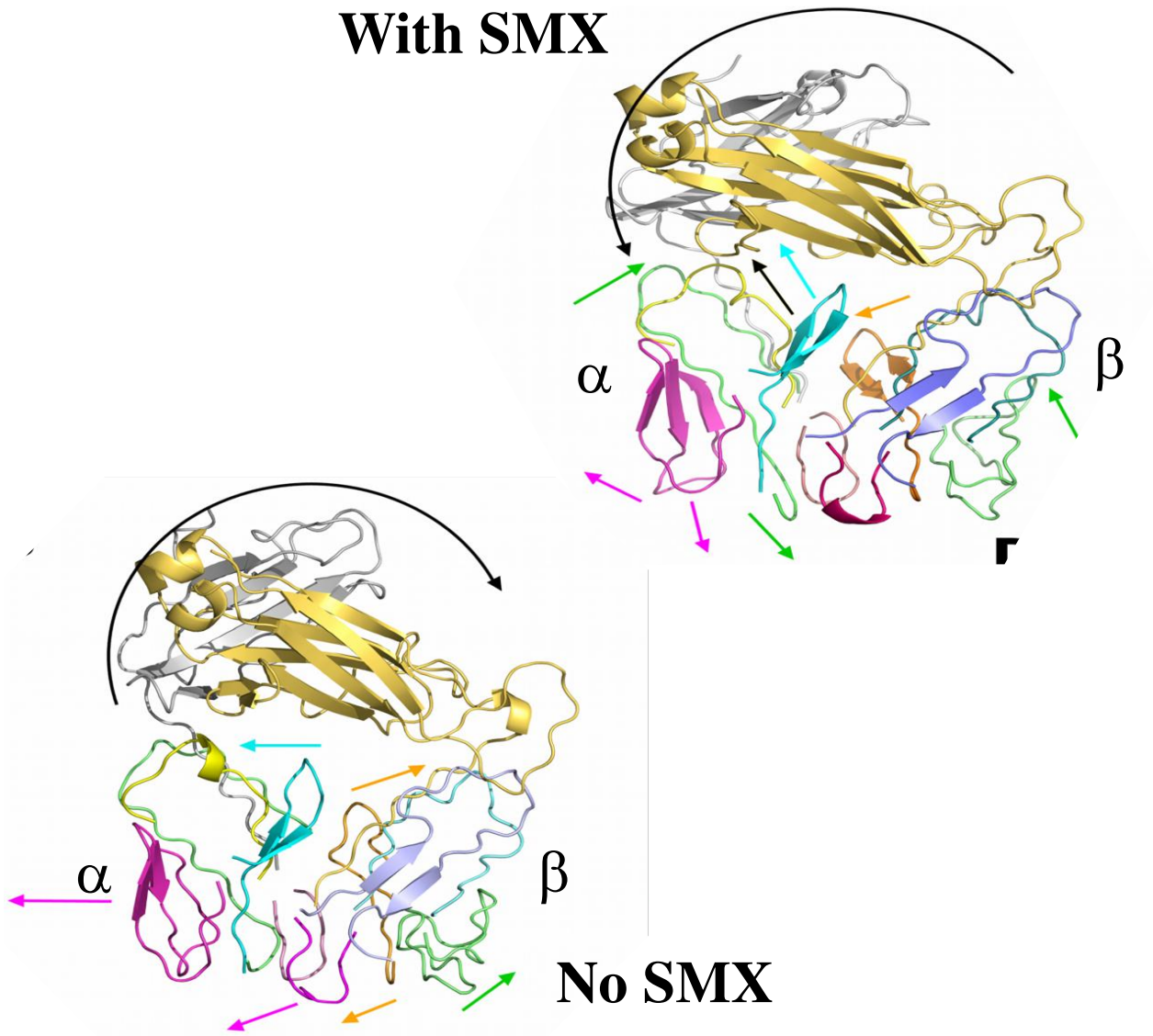




MD simulations of TCR H13 and HLA-DR*10:01 with or without SMX binding

Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14: 33-38, 27-38.

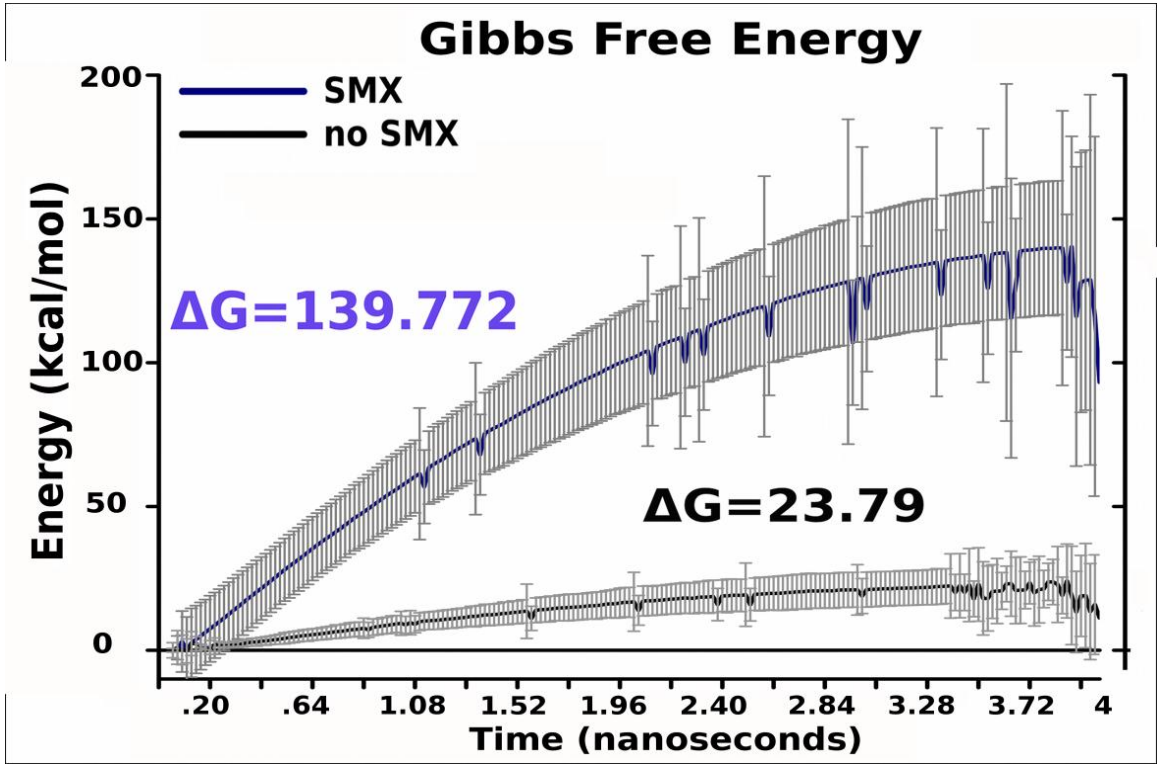
H13 Summary of Motions



With SMX:

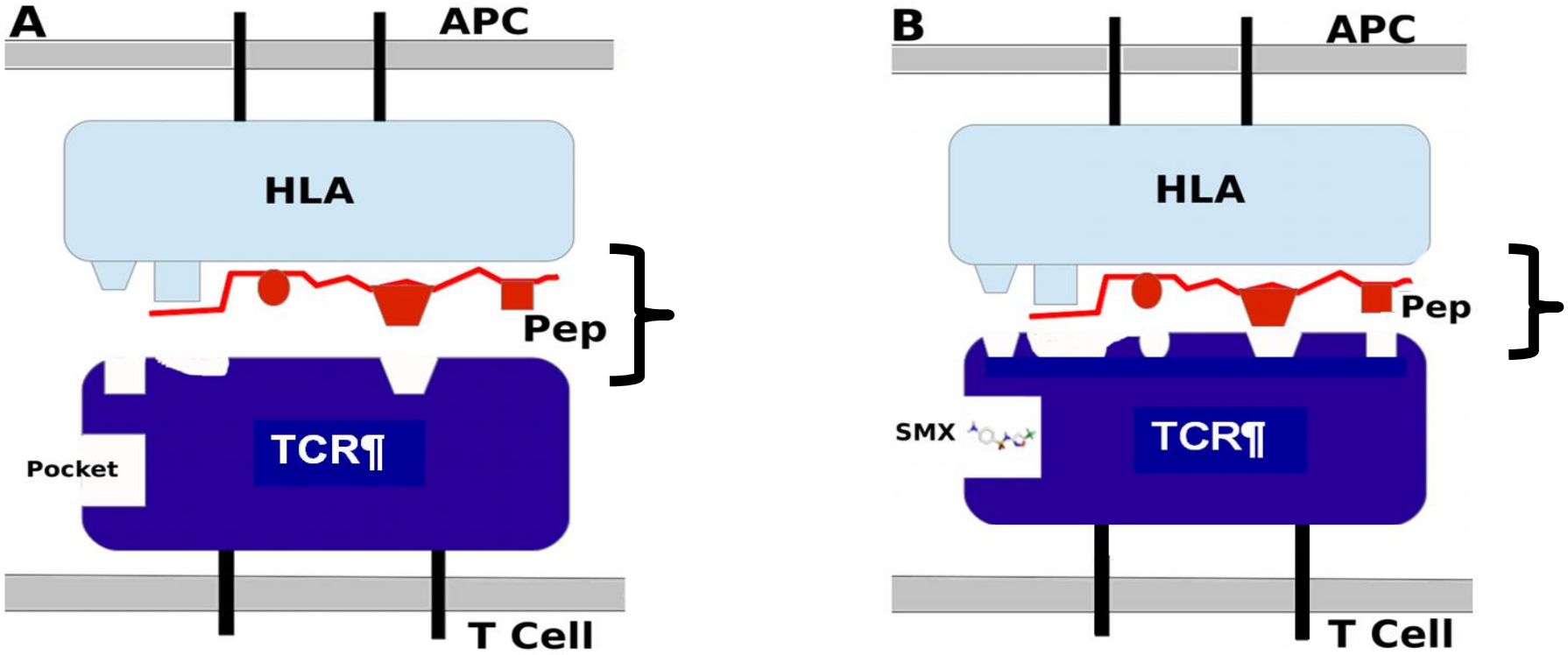
The analysis of motions reveals a “switch”, where the TCR constant domain either sits on the TCRVβ (above), changing to the TCRVα and a change from mostly Vβ recognition of the HLA and peptide, to a Vα recognition of the HLA.

Gibbs Free Energy, ΔG



- Free energy change is the most straightforward of the parameters
- For H13 it was shown SMX caused a **7 fold increase in affinity**, from -24 to -140 kcal/mol.
- This translates from a 2 µmol to a 0.79 µmol affinity.

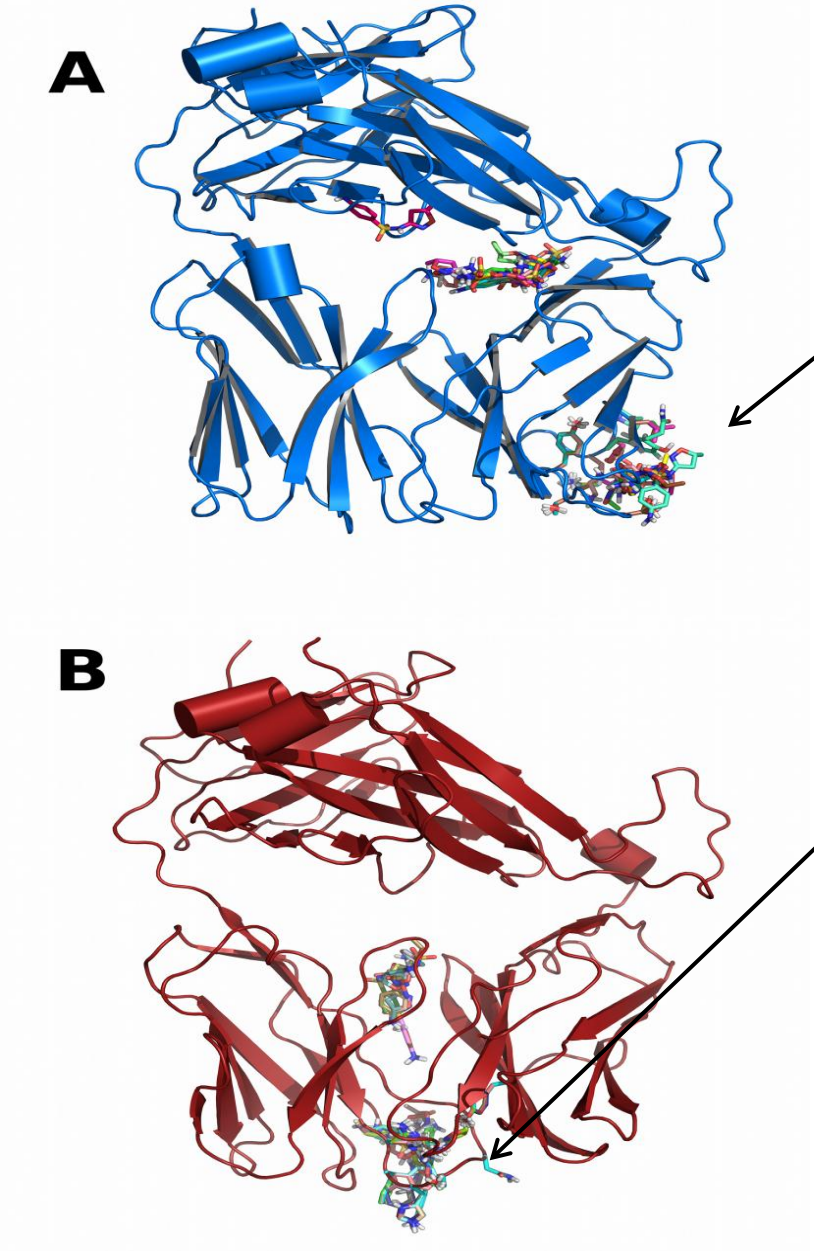
* Normal TCR affinities are in the range of 5-1 µmol, however we know the H13 T cell only proliferates with SMX present.



Allosteric effect of SMX binding to CDR2-Vβ pocket of TCR H13

Stephan Watkins & Werner J. Pichler: Sulfamethoxazole Induces a Switch Mechanism in T cell Receptors Containing TCRVβ-20-1 Altering pHLA Recognition, PLOS ONE, in press

understanding “p-i TCR”



H13

1.3

Two types of p-i TCR

A) A small molecule binds to a region on the TCR free from contact with the pHLA or other proteins-protein interfaces and the resulting complex can bind the pHLA through induced TCR conformations (*Watkins S & Pichler WJ, Plos One 2013*).

B) The CDR3α or β recognizes a small molecule, and the resulting complex can then bind the pHLA, with the small molecule acting as part of the TCR (*Watkins S & Pichler WJ, Open J Immunol, 2013)*.

-In either, there is a dependence on a particular pHLA, but the effect is mediated by the TCR binding the small molecule.

SUMMARY II: p-i concept

1. p-i: pharmacological interaction of drugs with immune receptors
2. one differentiates between p-i TCR and p-i HLA
3. It explains T-cell reactivity to drugs without implying antigenic features of the drug
4. most severe reactions appear to be due to **p-i,** which is an **off target** activity of the drug on (selected) immune receptors (TCR, HLA)
5. **In contrast to previous beliefs,** an interaction of small molecules with the immune system is common, and needs to be better investigated

6th DRUG HYPERSENSITIVITY MEETING (DHM6)

in

BERN, SWITZERLAND

APRIL 9th – 13th, 2014

www.eaaci.org

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Adverse Drug Reactions - Analysis & Consulting

SCAHT

Literature to drug eruptions

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